TARGETED ANTI-TNF THERAPY IN SEVERE SARCOIDOSIS:

TOWARDS PRECISION MEDICINE

Heleen Annette Crommelin

Targeted anti-TNF therapy in severe sarcoidosis: towards precision medicine
H.A. Crommelin

Thesis University of Utrecht

ISBN: 978-90-393-6993-7
Cover design and layout: evelienjagtman.com
Print: Gildeprint, Enschede

The research described in this thesis was performed with funding by St Antonius Hospital Innovation Fund.

TARGETED ANTI-TNF THERAPY IN SEVERE SARCOIDOSIS:
TOWARDS PRECISION MEDICINE

Gerichte anti-TNF therapie bij ernstige sarcoïdose: richting therapie op maat
(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 dinsdag 29 mei 2018 des middags te 2.30 uur

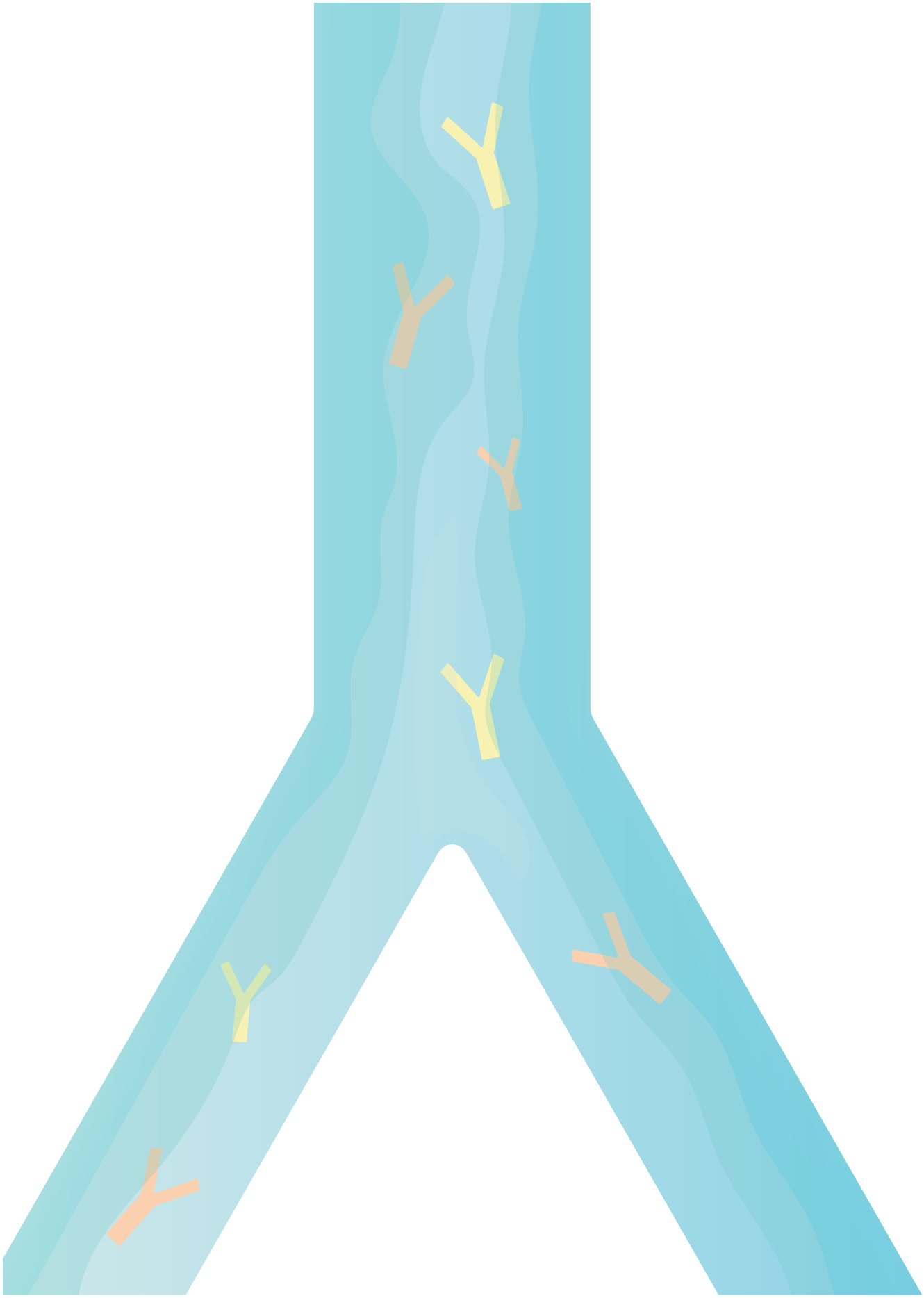
door

Heleen Annette Crommelin

geboren op 30 juli 1987
te Utrecht

Promotor:	Prof. dr. J.C. Grutters
Copromotoren:	Dr. V.H.M. Deneer
	Dr. C.H.M. van Moorsel

CONTENTS



1

Introduction and outline of the thesis

INTRODUCTION

Sarcoidosis is a granulomatous disease of unknown cause with a wide variety in disease expression. Most often pulmonary and lymphatic involvement is present, but eyes, skin, heart and nervous system are often involved as well. In rare cases the liver, central nervous system or bones may be affected. Symptoms depend on organ involvement, disease duration, ethnicity and activity of the granulomatous process. Diagnosis is established based on clinical and radiological findings and preferably histological evidence of non-necrotizing granulomas with exclusion of other known causes of granuloma formation. The clinical course of sarcoidosis shows wide variability, ranging from self-limiting to chronic and life-threatening. Sarcoidosis is a rare disease: incidence in The Netherlands is estimated to be 20 per 100,000 inhabitants and prevalence 50 per 100,000 inhabitants. Mainly patients in the age of 20 to 40 are first affected [1].

Disease etiology

Although the cause remains uncertain, it is assumed that sarcoidosis results from environmental exposure of a genetically susceptible host. The trigger is thought to be an antigen of yet unknown identity that is processed by antigen presenting cells (APCs) and presented to T lymphocytes. Upon release of stimulatory molecules, *e.g.* interleukin-2 (IL-2), tumor necrosis factor (TNF) and interferon-gamma (IFN-γ), activation and proliferation of T lymphocytes and monocytes occurs. Also other cells, such as B cells, are recruited. Granulomas are formed, consisting of epithelioid cells, giant cells and lymphocytes. TNF plays a pivotal role in this process of granuloma formation [1,2]. **Chapter 2** goes into more detail on the sarcoidosis etiology.

Pharmacological treatment

Since the cause of sarcoidosis is not known, curative pharmacotherapy is not available. However, immunosuppressive agents may control the symptoms of the disease. Main indications for pharmacological treatment are danger of organ failure and unacceptable loss of quality of life [3]. In approximately half of the patients with sarcoidosis the disease is not self-limiting and pharmacological treatment is required [4].

First- and second-line pharmacological treatment

Corticosteroids are considered to be first-line therapy, but their long-term use is hampered by side effects, such as osteoporosis, diabetes and weight gain. The optimal dose and duration of corticosteroid treatment remains a matter of debate. In case of insufficient effect, relapse or severe side effects, second-line therapy with cytotoxic agents is indicated. Most often a step-up approach is used because of the slow onset of effect of the cytotoxic agents: the cytotoxic agent is added to the corticosteroid treatment scheme and the corticosteroid is then later tapered. Most often cytotoxic agents such as methotrexate, azathioprine and leflunomide are chosen [5].

In some specific cases, agents that reduce inflammation by lowering TNF production (apremilast, pentoxyfilline and hydroxychloroquine) are also prescribed. However, these agents are used less often because of an unfavorable benefit-risk ratio. Available evidence on treatment of sarcoidosis with these non-targeted TNF inhibitors is described in more detail in **chapter 2**.

Third-line pharmacological treatment: specifically targeting TNF

There is a subgroup of patients where first- and second-line pharmacological treatment is ineffective or where side effects leading to discontinuation occur. This group of patients is estimated to comprise approximately 10% of all sarcoidosis patients and is referred to as patients with refractory sarcoidosis [4]. For these patients agents specifically inhibiting TNF are a third-line treatment option. Refractory sarcoidosis requiring anti-TNF therapy is a rare disease. As in most rare diseases, studies in this population are characterized by small sample sizes and single arm designs. Other factors that have impeded high-quality research in severe sarcoidosis are the heterogeneous clinical presentation and absence of a consensus on endpoints for evaluating outcomes. Guidelines on the anti-TNF treatment of sarcoidosis are based on limited clinical data and data obtained from other immune-mediated inflammatory diseases such as rheumatoid arthritis and Crohn's disease. Of the immune-mediated inflammatory diseases, Crohn's disease shows the highest resemblance to sarcoidosis: it is also a systemic, inflammatory disorder of unknown cause characterized by non-necrotizing granuloma formation. The peak onset is around the same age, between 20 to 40 years, and it is also a highly debilitating disease [6].

The anti-TNF agent that has been studied most thoroughly in refractory sarcoidosis is infliximab, a monoclonal antibody. However, conflicting results on efficacy of infliximab have been reported [7-10]. For adalimumab, another monoclonal TNF inhibitor, there is less clinical data [11,12]. Etanercept was found to be ineffective in sarcoidosis, as in other granulomatous disorders [13]. Recently, golimumab was also deemed ineffective in sarcoidosis [14]. Available evidence on the benefits and risks of anti-TNF agents in sarcoidosis and the presumed mechanism of action is presented in more detail in **chapter 2**.

Towards precision medicine

Currently, it is unknown what the optimal pharmacological treatment for each individual patient is in the treatment of severe sarcoidosis. In other words, there is a need for insights to apply precision medicine in these patients. The term precision medicine has been described extensively over the last few years. Jameson *et al.* [15] proposed the following definition: "We define precision medicine as treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations. Inherent in this definition is the goal of improving clinical outcomes for individual patients and minimizing unnecessary side effects for those less likely to have a response to a particular treatment".

Consensus on how to evaluate the response to pharmacological treatment in sarcoidosis is lacking. Various endpoints have been applied across clinical studies. Both in pulmonary and in extrapulmonary sarcoidosis several endpoints for the use in clinical practice have been proposed. But none of them has made its way into general clinical practice [16,17]. In **chapter 3** we study the effectiveness of infliximab in refractory sarcoidosis in a prospective, single arm study. We included inflammatory biomarkers and propose a composite score to evaluate response in the patient population of refractory sarcoidosis.

Previous studies have demonstrated that the risk of relapse is high after discontinuation of infliximab. Therefore, many patients are treated with infliximab for years. Very limited data are available on the benefit of infliximab in long-term treatment. In **chapter 4** we further address this issue.

In clinical practice adalimumab has been used as an alternative to infliximab in patients who initially responded to infliximab but became intolerant, typically because of loss of response and infusion reactions because of antibodies against infliximab. But no clinical data on whether adalimumab is an effective and safe alternative in these patients is available. **Chapter 5** describes efficacy and safety of adalimumab in this patient population.

Response to infliximab has shown great variability, in sarcoidosis and in other immune-mediated inflammatory diseases as well. Therefore, there is a clinical need for biomarkers that predict response. Genetics has been postulated as a possible factor influencing response to biologicals specifically targeting TNF. In **chapter 6** we tested whether genetic variations in TNF, TNF receptors and Fcγ-receptors that have been associated with response to infliximab in rheumatoid arthritis and inflammatory bowel disease are associated with response to infliximab in severe sarcoidosis. We also tested genetic variants in *HLA-DRB1* that have been linked with the occurrence of two different sarcoidosis phenotypes.

Finally, in **chapter 7** we developed a population pharmacokinetic model using NONMEM to describe the pharmacokinetics of infliximab and to study whether the infliximab concentration could serve as a predictor of response.

OBJECTIVE

The objective of this thesis was to study effectiveness of infliximab and adalimumab in severe sarcoidosis and to assess whether pharmacogenetics, inflammatory biomarkers, pharmacokinetics and infliximab exposure can aid in more precision based anti-TNF treatment in severe sarcoidosis.

OUTLINE OF THE THESIS

Chapter 2 provides an overview of the current knowledge on anti-TNF therapeutics in sarcoidosis.

Chapter 3 describes the short-term treatment outcomes of the first prospective, single arm study of infliximab in sarcoidosis with severe and active disease.

Chapter 4 describes the effect of infliximab in long-term treatment in severe sarcoidosis.

In **Chapter 5** the effect of adalimumab was studied in sarcoidosis patients who responded to infliximab but became intolerant and where the switch to adalimumab was made.

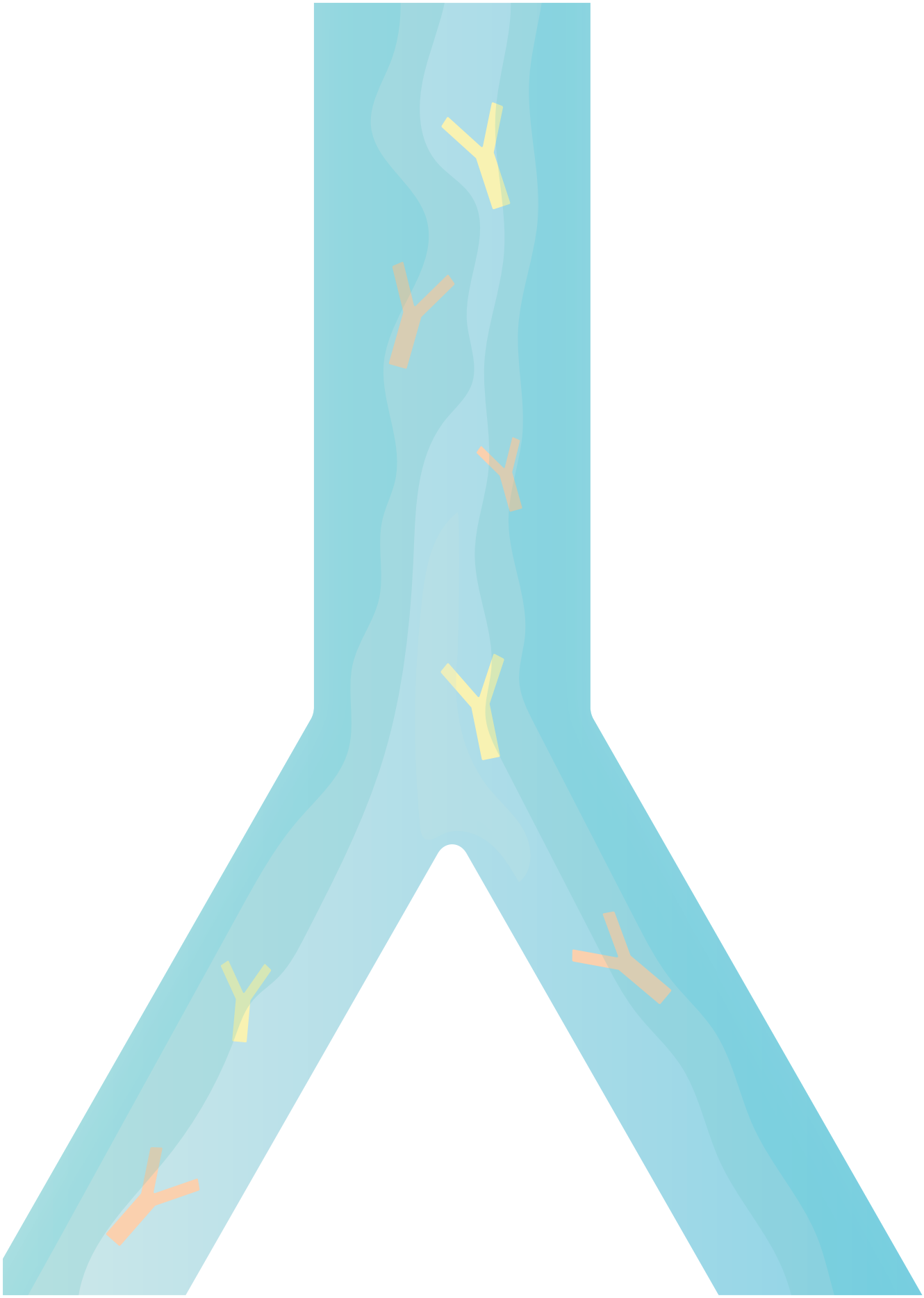
Chapter 6 shows data on genetic variations in *TNF, TNFRSF1A, TNFRSF1B, FCGR2A, FCGR3A* and tags for HLA-DRB*0301 and HLA-DRB*1501 in relation to the response to infliximab treatment.

In **chapter 7** a population pharmacokinetic model describing the pharmacokinetic characteristics of infliximab in patients with severe sarcoidosis is developed.

Chapter 8 summarizes the results of this thesis; it is followed by a general discussion, a section on future perspectives and concluding remarks.

REFERENCES

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
2. Chiarchiaro J, Chen BB, Gibson KF. New molecular targets for the treatment of sarcoidosis. *Curr Opin Pulm Med* 2016; 22: 515-521.
3. Judson MA. An approach to the treatment of pulmonary sarcoidosis with corticosteroids: the six phases of treatment. *Chest* 1999; 115: 1158-1165.
4. Nederlandse Vereniging van Artsen voor Longziekten en Tuberculose. Position paper: Behandeling van sarcoidose met TNF-alpha blokkerende middelen. 2012: 1-11.
5. Brito-Zeron P, Perez-Alvarez R, Pallares L, Retamozo S, Baughman RP, Ramos-Casals M, SarcoGEAS-SEMI Study Group. Sarcoidosis: an update on current pharmacotherapy options and future directions. *Expert Opin Pharmacother* 2016; 17: 2431-2448.
6. Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkuhn T, Orchard T, Rogler G, Louis E, Kupcinskas L, Mantzaris G, Travis S, Stange E, European Crohn's and Colitis Organisation (ECCO). The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010; 4: 7-27.
7. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
8. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller Jr W, Sands BE. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006; 23: 201-208.
9. van Rijswijk HNAJ, Vorselaars ADM, Ruven HTJ, Keijsers RGM, Zanen P, Korenromp IHE, Grutters JC. Changes in disease activity, lung function and quality of life in patients with refractory sarcoidosis after anti-TNF treatment. *Expert Opinion on Orphan Drugs* 2013; 1: 437-443.
10. Hostettler KE, Studler U, Tamm M, Brutsche MH. Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218-224.
11. Pariser RJ, Paul J, Hirano S, Torosky C, Smith M. A double-blind, randomized, placebo-controlled trial of adalimumab in the treatment of cutaneous sarcoidosis. *J Am Acad Dermatol* 2013; 68: 765-773.
12. Erckens RJ, Mostard RL, Wijnen PA, Schouten JS, Drent M. Adalimumab successful in sarcoidosis patients with refractory chronic non-infectious uveitis. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 713-720.
13. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest* 2003; 124: 177-185.
14. Judson MA, Baughman RP, Costabel U, Drent M, Gibson KF, Raghu G, Shigemitsu H, Barney JB, Culver DA, Hamzeh NY, Wijsenbeek MS, Albera C, Huizar I, Agarwal P, Brodmerkel C, Watt R, Barnathan ES. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. *Eur Respir J* 2014; 44: 1296-1307.
15. Jameson JL, Longo DL. Precision medicine - personalized, problematic, and promising. *N Engl J Med* 2015; 372: 2229-2234.
16. Baughman RP, Nagai S, Balter M, Costabel U, Drent M, du Bois R, Grutters JC, Judson MA, Lambiri I, Lower EE, Muller-Quernheim J, Prasse A, Rizzato G, Rottoli P, Spagnolo P, Teirstein A. Defining the clinical outcome status (COS) in sarcoidosis: results of WASOG Task Force. *Sarcoidosis Vasc Diffuse Lung Dis* 2011; 28: 56-64.
17. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, Drent M, Centocor T48 Sarcoidosis Investigators. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J* 2008; 31: 1189-1196.



2

Anti-TNF therapeutics for the treatment of sarcoidosis

Heleen A Crommelin
Adriane DM Vorselaars
Coline HM van Moorsel
Ingrid HE Korenromp
Vera HM Deneer
Jan C Grutters

Immunotherapy. 2014;6(10):1127-1143.

ABSTRACT

Sarcoidosis is a systemic disease with an incidence of 1 to 40 per 100,000 persons per year. It predominantly affects people in the age of 20 to 40 years old. Disease course varies from mild self-limiting to chronic debilitating and life-threatening disease. Since the cause of sarcoidosis is unknown, curative therapy is not available. Immunosuppressive drugs may, however, control the symptoms of the disease. The hallmark of sarcoidosis is the formation of granulomas that are most commonly found in lungs and lymph nodes. As TNF plays an important role in both formation and maintenance of these granulomas, as well as in the immune response, anti-TNF biologicals such as infliximab and adalimumab are considered a last resort therapeutic option. Clinical effectiveness, however, varies considerably and data showing which patients would benefit most from this expensive therapy are scarce. This review summarizes current knowledge on anti-TNF therapeutics in sarcoidosis, and describes insights into prediction of response, outcome measures and antibody development.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology that can present in many clinical phenotypes, ranging from asymptomatic and self-limiting to severe and life-threatening disease [1-4]. Pulmonary involvement is seen in approximately 90% of cases, but often other organs such as lymph nodes, eyes and skin are involved [2,5-7]. Diagnosis is established favorably on histological evidence of non-caseating epithelioid cell granulomas in the involved organs together with exclusion of other causes for granulomatous disease [2,8,9].

Sarcoidosis has an incidence of 1 to 40 per 100,000 persons per year and most often affects patients aged 20 to 40 years old [2,10]. It occurs in both sexes, although slightly more often in women than in men [11-13]. Spontaneous resolution of the disease occurs in approximately two-thirds of patients. In 10 to 30% of patients, the disease is chronic with progressive organ failure [2,14]. Sarcoidosis is found in people from all genetic backgrounds, although there are striking differences in incidence and severity across ethnicities. For instance, prevalence of sarcoidosis is higher in African-Americans and they are also often found to have more severe disease than Caucasians [13,15-17]. Furthermore, localization of the disease varies between different genetic backgrounds: Japanese have a relatively higher risk of developing ocular and cardiac sarcoidosis [12], whereas the acute form of sarcoidosis with a good prognosis, also known as Löfgren's syndrome, is more often found in Caucasians [18,19]. Mortality in sarcoidosis is higher than in the general population. In western countries most cases of death are due to respiratory complications [20,21]. However, in Japanese patients mortality is most often due to cardiac involvement [22,23]. Mortality rates at national level have been described in the United States and Japan between 1972 and 1991, with an age-adjusted mortality rate of 1.6-2.1 per million in the United States and 0.1-0.2 per million population in Japan [20,21]. Overall, mortality in sarcoidosis ranges from 1 to 5% of patients [2,24].

Immunologically, sarcoidosis is believed to start with the interaction between an antigen-presenting cell and an unknown antigen. This antigen is presented to naive CD4+ lymphocytes (Th0) and alveolar macrophages and both cell types are activated. Further activation and proliferation of these cells occurs upon release of stimulatory molecules, for example, interleukin 2 (IL-2), tumor necrosis factor (TNF) and interferon-gamma (IFN-γ), by both cell types [25-28]. In chronic disease only alveolar macrophages may be activated, resulting in lower numbers of helper T cells and eventually leading to fibrosis [29-31]. It is believed that due to the ongoing presence of the antigen, ultimately non-caseating granulomas are formed. In both manifestations of sarcoidosis, TNF plays a critical role, both during immune response and in the formation of granulomas [2,29,30] (Figure 1). Granulomas are pathological entities that comprise macrophages and T lymphocytes and can cause organ damage, ultimately leading to dysfunction [1]. These granulomas are also found in infectious diseases such as tuberculosis, and are a protective mechanism against infections [32,33]. However, in sarcoidosis an infectious cause has not been found so far. Therefore, it remains unclear whether incessant granuloma formation is a response to an (ongoing) unidentified infectious trigger or a derailed autonomous immunopathological process [30,34].

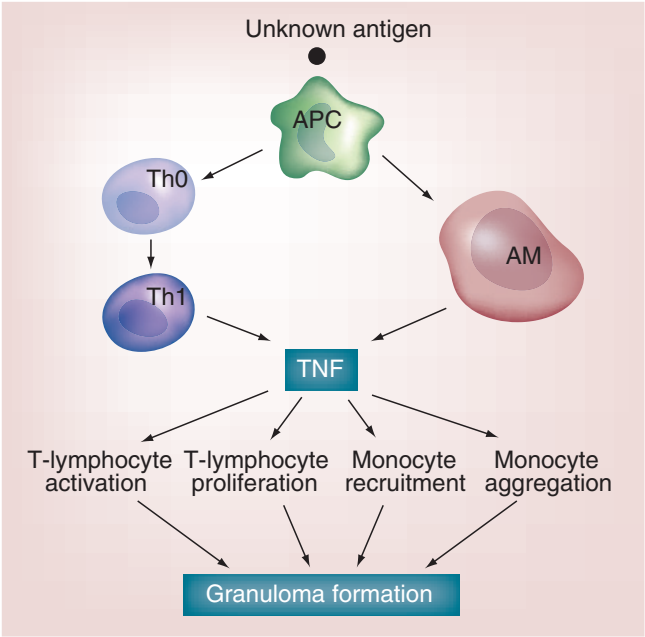


Figure 1. Role of TNF in granuloma formation in sarcoidosis.
An unknown antigen activates naive T cells and alveolar macrophages. These cells produce TNF and other chemokine ligands, resulting in activation and proliferation of T lymphocytes and recruitment and aggregation of monocytes. Ongoing presentation of the antigen ultimately leads to formation of non-caseating granulomas. AM: alveolar macrophage; Th0: naive CD4+ T lymphocyte; Th1: T helper 1 cell.

Although there may be an unidentified environmental trigger, genetic predisposition may also play a role. A genetic component to the disease has been described by means of familial clustering [35,36]. In fact, genetic studies have shown that sarcoidosis disease susceptibility is caused by a combination of genes that have been previously found to be associated with a wide variety of immune-mediated inflammatory diseases (IMIDs) [37]. At present, the disease is conceptualized as a complex disease caused by an environmental trigger in a genetically susceptible individual [38]. This trigger, however, remains elusive. Because the lungs, skin and eyes are most often involved in sarcoidosis, it is thought that the cause might be airborne [1].

Because of the unknown etiology of sarcoidosis, therapy is not directed towards the cause of the disease. Instead, current aim of sarcoidosis treatment is inhibiting the activity of the immune system in this disease. Therapeutic curing of the disease is not thought possible, so treatment is directed towards reducing inflammatory symptoms [39].

In the majority of patients, treatment is not needed due to the remissive nature of the disease [1,2,14]. When treatment is indicated, generally, the first step in therapy is corticosteroids [40,41]. Local therapy with corticosteroids can be attempted in sarcoidosis with skin or eye localization [2]. Inhaled corticosteroids may improve bronchial hyperreactivity, but are not known to reduce pulmonary granuloma formation [42,43]. Severe pulmonary sarcoidosis or involvement of heart

and (central) nervous system can only be treated by systemic therapy [2]. When the effect of systemic therapy with corticosteroids is insufficient or has significant side effects, such as obesity or diabetes, a second-line therapeutic is indicated [44]. Methotrexate is commonly seen as the first drug of choice in second-line therapy. Other options include azathioprine, leflunomide or hydroxychloroquine. Only if second-line therapy fails or one of the available drugs is contra-indicated, for example, methotrexate in case of severe liver function impairment, TNF blocking biologicals are considered as a last resort treatment option [44,45]. However, due to the rarity of the disease and limited availability of data from clinical trials, currently no guidelines on how and when to prescribe anti-TNF therapy exist. Furthermore, none of the anti-TNF therapeutics is officially registered for use in sarcoidosis.

Hence, this review focuses on the current knowledge on anti-TNF therapeutics in sarcoidosis. We will highlight the different drugs and their effectiveness.

Rationale for inhibiting TNF in sarcoidosis

In general, the cytokine TNF (formerly also known as TNF-α) is a homotrimeric protein that plays an important role in the pro-inflammatory cascade. Its name 'tumor necrosis factor' refers to its tumoricidal effect when injected in mice [46]. It is produced by both immune and nonimmune cell types, including macrophages, T cells, granulocytes, fibroblasts and smooth muscle cells [47]. When binding to its receptor, TNF initiates cell proliferation or apoptosis [48].

Apart from the key role in the immunological response, TNF is also a crucial factor in the formation of granulomas. *In vivo* experiments with mice give evidence for the latter critical role. Intratracheal injection of TNF in mice induced the formation of epithelioid granulomas composed of macrophages, occasional giant cells and neutrophils [49]. Also, in genetic studies, *TNF* has been associated with the development of granulomas in mice: *TNF* knockout mice appeared to be less prone to develop granulomas than control mice [50,51].

In sarcoidosis, the role of TNF has become clear by multiple studies. Foremost, increased levels of TNF were detected in bronchial alveolar lavage (BAL) fluid of sarcoidosis patients. It is believed that this excess TNF is produced by macrophages [30]. In addition, *in vitro* experiments with BAL from pulmonary sarcoidosis patients showed that macrophages in this BAL were more prone to produce TNF, both spontaneously and when induced by lipopolysaccharides (LPS) [26-28]. Furthermore, TNF release by BAL obtained alveolar macrophages showed that high levels of spontaneous TNF release at the time of diagnosis correlated with a significantly higher risk for disease progression [52].

In addition to the increased levels of TNF in BAL, upregulated concentrations of TNF are also found in the blood of sarcoidosis patients [53]. Plasma concentrations of TNF in patients with sarcoidosis were 42% higher than in healthy control subjects [54].

Also, genetic studies have been performed on the association between polymorphisms in the *TNF* gene and susceptibility to sarcoidosis. Different phenotypic presentations of sarcoidosis have been reported to associate with *TNF* polymorphisms [55-57]. For instance, carriership of the

TNF -308A allele is associated with enhanced production of TNF, higher susceptibility to sarcoidosis and a persistent disease phenotype [58,59]. However, these results should be interpreted with caution, because they might be significantly influenced by the strong linkage between TNF and the nearby-situated *HLA-DRB1* gene [59]. Alleles of *HLA-DRB1* are strongly related with susceptibility and disease development in sarcoidosis [60]. Therefore, the reported association between *TNF* -308A and sarcoidosis could be caused by linkage to variation in *HLA-DRB1*.

All in all, we conclude that TNF is a critical factor in sarcoidosis, both for the immune response and the formation of granulomas. Inhibiting the effect of TNF by immunomodulatory therapy is therefore a logical proposition.

ANTI-TNF THERAPEUTICS IN SARCOIDOSIS

In sarcoidosis, two different types of anti-TNF drugs are used. First of all, there are the immunomodulatory drugs with an inhibitory effect on TNF-production: thalidomide, pentoxifylline and apremilast. They do not directly target TNF production, but are aimed at enzymes such as phosphodiesterase 4 (PDE4) or cyclic adenosine monophosphate (cAMP). In doing so they also inhibit production of other cytokines such as IL-6, IL-12 and IFN-γ. These non-targeted anti-TNF drugs have been studied in different manifestations of sarcoidosis with variable results. So far, prescription of these drugs in clinical practice is very uncommon, likely due to the limited positive data and clinical experience together with an unfavorable risk/benefit ratio. The second type of anti-TNF drugs works through targeted inhibition. These drugs are derived from living cells using recombinant DNA technology and are therefore called biologicals. They are designed to specifically suppress response to TNF. The anti-TNF biologicals that have been studied for treatment of sarcoidosis are infliximab, adalimumab and etanercept. They are currently not considered first-line therapy in sarcoidosis, but usually they are given when more than one of the other treatment options have failed.

The next section will discuss clinical effectiveness of the non-targeted inhibitors of TNF production and of the anti-TNF biologicals in sarcoidosis (Table 1), followed by a discussion on the molecular differences between these anti-TNF biologicals that might account for differences in clinical response.

Non-targeted inhibition of TNF production

This paragraph highlights the three immunomodulatory drugs with a non-targeted inhibitory effect on TNF production: thalidomide, pentoxifylline and apremilast.

Thalidomide

Thalidomide is an immunomodulating drug that reduces TNF production and excretion by alveolar macrophages [61,62], NK cells and T lymphocytes [63]. Thalidomide was originally introduced as a sedative, but its use has been limited due to its well-known teratogenic properties. The

mechanism of thalidomide in sarcoidosis is unknown and no randomized controlled trials (RCTs) on this subject have been published in sarcoidosis. Nevertheless, evidence for positive results for thalidomide derive from pilot trials and case series. First of all, in an open-label dose-escalation trial with a selected group of patients with chronic cutaneous sarcoidosis all patients (n=14) experienced subjective response after four months of treatment. Objectively, the blinded photograph scoring showed improvement in ten out of twelve evaluable patients as well [64]. Additionally, in a case series with patients with cutaneous sarcoidosis, two out of twelve patients had complete regression of all cutaneous lesions after treatment with thalidomide. In six patients the lesions had decreased but without complete regression and two other patients showed no regression but only worsening. Common side effects were somnolence and numbness [65]. In pulmonary sarcoidosis, two studies investigating thalidomide have been conducted. The first study was an open-label trial in ten patients with corticosteroid-dependent pulmonary sarcoidosis. Response was evaluated after 24 weeks of treatment with 200 mg thalidomide per day. After twelve weeks of treatment, baseline corticosteroid dose was reduced by 50% as part of the study protocol. No significant changes in spirometry as measured by forced vital capacity (FVC), quality of life as measured by the Short Form-36 and dyspnea as measured by the Translational Dyspnea Index were found in this study. Remarkably, nine out of the ten patients required dose reduction of thalidomide due to sedation and paresthesia, although no severe side effects were reported [66]. A second study, however, did demonstrate a favorable effect of thalidomide in patients with pulmonary and cutaneous sarcoidosis (n=19). Patients initially received a dose of 200 mg/day, the same as in the afore-mentioned study, but with dose reduction after two weeks of treatment to 100 mg/day and further reduction at week twelve. After six months of treatment, skin lesions were resolved in 67% of patients. In addition, soluble angiotensin I converting enzyme (sACE) levels decreased significantly during treatment, especially in the first three months, reaching levels within the normal range in 74% of patients at that time point. Moreover, radiology showed improvement after six months of treatment. However, spirometry showed no significant changes, although the diffusing capacity of the lungs for carbon monoxide (DLCO) showed a trend towards improvement. Response on cutaneous lesions, an increase in DLCO and a larger reduction in sACE were inversely correlated with disease duration. The authors suggested their more complete and systemic evaluation of the pulmonary effect as a plausible cause for the discrepancy in efficacy between the two studies. Apart from the earlier mentioned side effects of somnolence and numbness, 42% of patients developed peripheral neuropathy in this study [63]. Nevertheless, in pulmonary sarcoidosis low doses of thalidomide are often not effective for parenchymal involvement and side effects appear to be dose dependent [66]. Therefore, thalidomide is not prescribed very frequently. However, in selected cutaneous and refractory sarcoidosis patients with internal organ involvement the addition of thalidomide to the prescription regimen may be worthwhile to consider [67].

Pentoxifylline & apremilast

Alternative immunomodulatory drugs with a non-targeted inhibitory effect on TNF production are pentoxifylline and apremilast. These phosphodiesterase inhibitors reduce TNF release by alveolar macrophages as well. Competitive inhibition of phosphodiesterase type 4 raises intracellular cAMP, leading to down regulation of TNF production [68-70]. The effect of pentoxifylline was investigated in two studies. In the first study in patients with pulmonary sarcoidosis, pentoxifylline treatment improved DLCO and arterial oxygen tension (PaO2) on exercise, especially in steroid-naive patients [71]. In patients who were already on systemic corticosteroid treatment, combination therapy with pentoxifylline resulted in the tapering of the daily dosage of prednisone [71]. Secondly, an RCT in patients with pulmonary sarcoidosis (n=27) reported a significant steroid-sparing effect after eight and ten months of therapy. The mean prednisone dose in the pentoxifylline group of 15 mg/day was reduced to 0.5 mg/day after ten months of therapy. This reduction was larger than that observed in the placebo group (16 to 9 mg/day) [72], indicating that adding pentoxifylline has a steroid-sparing effect.

Table 1. Anti-TNF drugs in sarcoidosis therapy.

Drug	***Indication most often prescribed for***	***Half-life***	***Adverse effects***	***Drug Class***
Thalidomide	Multiple myeloma[b]	5.5-7.3 h	Neutropenia, leukopenia, constipation, somnolence, numbness	Immunomodulatory agent
Pentoxifylline	Intermittent claudication in peripheral artery disease	0.4-0.8 h	Nausea, dizziness	Phosphodiesterase inhibitor
Apremilast	Ankylosing spondylitis, Ps and PsA	8.2 h	Headache, nausea, upper respiratory infection	Phosphodiesterase inhibitor
Etanercept	RA	70 h	Infections	Fusion protein
Infliximab	RA and IBD	8-9.5 days	Infections, tuberculosis	mAb
Adalimumab	RA and IBD	14 days	Infections	mAb

[a] Maintenance dose
* Based on data from randomized controlled trials and large cohort studies
[b] Due to its teratogenic properties thalidomide should be prescribed with caution

Finally, the effect of apremilast was evaluated in a single study. The study comprised fifteen patients with chronic cutaneous sarcoidosis, mainly black women. Response to apremilast was determined by Sarcoidosis Activity and Severity Index (SASI) and comparison of photographs of the index lesions before and after twelve weeks of treatment. Both SASI and photographic score showed statistically significant improvement after therapy. However, skin lesions worsened significantly in three patients within three months after cessation of apremilast treatment [73]. So, pentoxifylline showed some steroid-sparing effect and apremilast showed improvement in chronic cutaneous sarcoidosis, but both were studied in only a small number of patients.

In conclusion, there is little evidence for a beneficial effect of non-targeted inhibitory TNF drugs in sarcoidosis and these agents are used infrequently. However, there might be some niche indication such as refractory severe skin sarcoidosis.

Administration route + dose[a]	***Studies performed in sarcoidosis***	***Current status for indication in sarcoidosis****
Oral, 100 or 200 mg/day	Baughman *et al.* [1] Nguyen *et al.* [2] Judson *et al.* [3] Fazzi *et al.* [4]	Cutaneous Pulmonary[c]
Oral, 25 mg/kg/day	Zabel *et al.* [5] Park *et al.* [6]	Pulmonary
Oral, 20 mg/day	Baughman *et al.* [7]	Cutaneous
Subcutaneously, 25 mg twice weekly	Utz *et al.* [8] Baughman *et al.* [9]	-
Intravenously, 5 mg/kg bodyweight with interval of 4 weeks	Rossman *et al.* [10] Baughman *et al.* [11] Van Rijswijk. [12] Judson *et al.* [13] Hostettler *et al.* [14] Russell *et al.* [15]	Severe pulmonary and extrapulmonary sarcoidosis patients refractory to first- and second-line treatment
Subcutaneously, 80 mg/week	Pariser *et al.* [16] Erckens *et al.* [17] Sweiss *et al.* [18]	Cutaneous Non-infectious uveitis Refractory pulmonary

[c] Conflicting study results

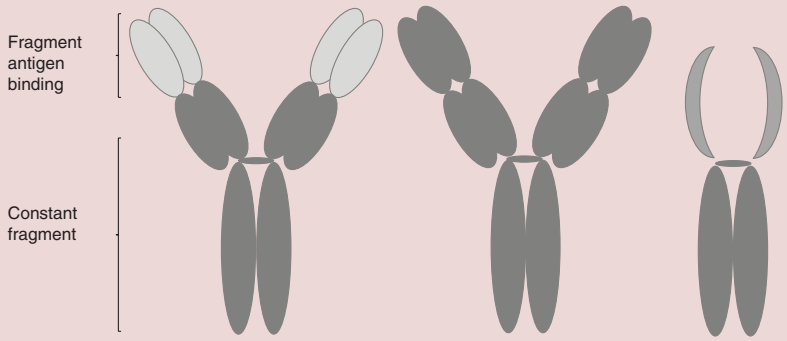
IBD: inflammatory bowel disease; mAb: monoclonal antibody; Ps: psoriasis; PsA: psoriatic arthritis; RA: rheumatoid arthritis.

Targeted inhibition of TNF: biologicals

In addition to non-targeted inhibition, drugs that inhibit TNF in a targeted way have been evaluated in sarcoidosis. These drugs are derived from living cells using recombinant DNA technology and are therefore referred to as biologicals. In this section we will focus on infliximab, adalimumab and etanercept.

Infliximab

Infliximab is a chimeric monoclonal antibody, comprising murine variable parts and human constant IgG1 regions (Figure 2). The elimination half-life of infliximab is 8 to 9.5 days [74]. It is administered intravenously. The effect of infliximab in sarcoidosis has been tested in several RCTs. The very first published RCT (n=19) showed improvements of lung function upon treatment with infliximab [75]. Initially, the treatment arm received infliximab 5 mg/kg at weeks 0 and 2. At week 6, major endpoints were measured. Thereafter, all patients received open-label infusion of infliximab at week 6 and 14 and were followed up until week 38. After six weeks of treatment, the change in the infliximab arm in % predicted mean vital capacity (VC) was 5.1 versus 2.2 in the placebo arm. However, this difference was not statistically significant. After 22 weeks of infliximab treatment, the mean change in forced vital capacity (FVC) predicted was 6% [67,75]. In line with this study, a second double-blinded RCT in a larger cohort (n=126) of sarcoidosis patients, revealed increase in lung function as well [76]. Baughman *et al.* [76] compared two different drug regimens, infliximab 3 mg/kg and infliximab 5 mg/kg, and placebo. All patients received infusions at week 0, 2, 6, 12, 18 and 24. Patients with pulmonary and extrapulmonary sarcoidosis were both enrolled in the study. The mean increase from baseline in FVC in the infliximab arm was 2.5% predicted versus no change in the placebo group [76]. Although the increase in FVC was statistically significant between the two groups, the question arises whether an increase of 2.5% predicted in FVC might be clinically significant. Based on a *post hoc* analysis, the authors suggested, however, that patients with a lower FVC were more prone to have a larger improvement in FVC on infliximab treatment [67,76]. The latter could also explain the difference in change in FVC predicted between the studies by Rossman *et al.* [75] and Baughman *et al.* [76]: the patients in the first study had a lower mean FVC at baseline (59.63 vs 68.6% predicted). In patients with extrapulmonary involvement, the total score of extrapulmonary sarcoidosis severity expressed as ePOST, was decreased by more than 40% in the treatment group and differed significantly from the placebo group [77]. Another point of discussion deriving from this second RCT was whether a high prednisone dose might interfere with improvements in FVC [78]. An additional stratified analysis showed that infliximab treatment combined with a prednisone dose above 15-20 mg/day was associated with less improvement in FVC over a treatment period of 24 weeks compared to combined treatment with a lower dose of prednisone. It was suggested that corticosteroids may induce TNF suppression and that infliximab may therefore not be of additional significant benefit.



	Infliximab (Remicade®)	Adalimumab (Humira®)	Etanercept (Enbrel®)
Constant fragment	Human IgG1	Human IgG1	Human IgG1
Fragment antigen binding	Murine	Human	Human (TNFR2)

Figure 2. Anti-TNF biologicals used in sarcoidosis with an immunoglobulin G1 constant Fc fragment and a variable antigen-binding site. TNFR2: TNF receptor 2.

Apart from the RCTs, a retrospective study also described the effect of infliximab in sarcoidosis. In a cohort of 48 patients treated with six infusions of 5 mg/kg, infliximab showed a significant improvement of vital capacity (VC) and forced expiratory volume in one second (FEV1) in patients with a pulmonary treatment indication (7.6 and 7.9% predicted, respectively) after 18 weeks. Furthermore, in this study disease activity was not only evaluated by lung function parameters, but also by ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) scan. In the pulmonary parenchyma, the maximum standardized uptake value (SUVmax) significantly decreased by -2.7 as well as a significant decrease of -2.3 in the mediastinum. Interestingly, this study was the first to assess the positive effect of infliximab on quality of life [79]. Furthermore, two cohort studies on long-term treatment with infliximab in sarcoidosis patients who failed to respond to conventional therapy showed beneficial results for both patients with pulmonary and extrapulmonary sarcoidosis [80,81].

Finally, a substantial number of case reports and case series have reported positive treatment results of infliximab in extrapulmonary sarcoidosis, such as cardiac, neurological, osseous and ocular localizations [82-86].

Currently, infliximab is often administered at a dose of 5 mg/kg bodyweight every four weeks. This scheme is based on very limited data from sarcoidosis trials and on clinical experience. However, this treatment regimen has not been studied in an RCT or a large number of patients, leaving the optimal dosing regimen of infliximab in sarcoidosis still matter of debate.

In summary, there is evidence for a significant improvement of FVC in sarcoidosis patients treated with infliximab. However, the clinical relevance of this improvement is still unclear and infliximab is still not licensed for treatment of sarcoidosis. Recent studies show a clinically relevant effect in a subgroup of patients with more severe disease.

Adalimumab

An alternative targeted TNF inhibitor is adalimumab. Adalimumab is a fully human monoclonal antibody containing human constant IgG1 regions (Figure 2). While the variable regions of infliximab are murine, in adalimumab these regions are human. Adalimumab has an elimination half-life of approximately two weeks [87]. It is administered subcutaneously.

In sarcoidosis, the effect of adalimumab has been evaluated in three different study populations: cutaneous sarcoidosis, sarcoidosis patients with uveitis and refractory sarcoidosis. In cutaneous sarcoidosis a double-blinded RCT (n=16) showed positive results for the treatment group that received a loading dose of adalimumab of 80 mg subcutaneously, followed by a weekly dose of 40 mg. After 12 weeks, the target lesion area significantly decreased with an average of 32% versus an increase of 54% in the placebo arm [88]. Also, in sarcoidosis patients with refractory chronic noninfectious uveitis (n=26) treatment with adalimumab brought a reduction in disease activity. In 85% of patients, intraocular inflammatory signs as scored by an ophthalmologist improved after six months. In addition, biomarkers decreased significantly: mean sACE decreased from 19 to 14 U/L and mean soluble interleukin-2 receptor (sIL-2R) decreased from 2803 to 1888 pg/mL. After 12 months of treatment, mean sIL-2R of 2188 pg/mL was still significantly lower than at baseline, even though mean sACE of 15 U/L was not [89]. Third, in patients with refractory sarcoidosis the effect of a dose of 40 mg adalimumab subcutaneously every other week was evaluated by a prospective observational study (n=10). Although median SUVmax and mean standardized uptake values (SUVmean) were significantly reduced after 24 weeks of treatment (14.1 to 7.0 and 6.5 to 2.9, respectively), no changes were seen in pulmonary function tests or sACE [90]. The latter may be attributed to the fact that a lower dose of adalimumab was used compared to the afore-mentioned studies. Nevertheless, the authors suggest that adalimumab may have prevented further deterioration of lung function. Recently, an open-label study on the effect of adalimumab was performed in African-American women with refractory pulmonary sarcoidosis (n=11). After 24 weeks of treatment, nine patients showed a clinically significant response on reduction of concomitant immunosuppressive therapy, improvement of FVC and/or 6-minute walk test distance. Response was still present after 54 weeks in 8 out of 10 patients remaining in the study. Overall health status, reflected by the physician's and patient's global assessment score, showed improvement at weeks 24 and 54 compared to baseline [91].

A number of case reports described the use of adalimumab in different localizations of sarcoidosis such as osseous, ocular and bone marrow and also showed good treatment results [86,92,93].

In summary, current evidence for the effectiveness of adalimumab in sarcoidosis is limited. It is not licensed for the treatment of sarcoidosis. All the same, adalimumab shows beneficial effect in treatment of sarcoidosis.

Etanercept

The last targeted inhibitor of TNF is etanercept. Etanercept is a recombinant dimeric fusion protein of the human TNF receptor (TNFR) p75 (TNFR2) and the constant fragment of immunoglobulin (Ig) G1 (Figure 2). The drug contains the extracellular ligand-binding region of the receptor [94]. It is linked to the Fc-region of an IgG1 molecule, creating a synthetic TNFR with an elimination half-life of approximately four days. Etanercept is administered subcutaneously.

Etanercept has proven to be effective in treatment of rheumatoid arthritis [95,96]. However, administration of etanercept was found to be safe but not effective in patients with moderate-to-severe Crohn's disease [47,97]. Its use in pulmonary sarcoidosis was studied by Utz *et al*. [98] in a prospective open-label phase-2 treatment trial. Seventeen patients with pulmonary sarcoidosis Scadding stage II (bilateral hilar adenopathy with parenchymal infiltration) and III (parenchymal infiltration without hilar adenopathy) were included, but the study was terminated early because of excessive treatment failures. With two-thirds of patients not responding and some showing worsening of lung function, etanercept was not found to be effective in this study group [98]. Subsequently, a study in patients with refractory ocular sarcoidosis treated with etanercept showed neither significant improvement according to an ophthalmologist nor significant reduction in corticosteroid dosage [99]. Therefore, in sarcoidosis etanercept is regarded as an ineffective drug.

Biologicals against TNF in sarcoidosis: molecular differences

Although anti-TNF biologicals have shown effect both in sarcoidosis and in other IMIDs, the precise mechanism of action of these drugs remains unknown. Currently, it is believed that anti-TNF biologicals exert their effect by blocking pathways mediated by the TNF receptor (TNFR) or by inhibiting binding between the TNFR and membrane-bound TNF (tmTNF) [100]. Infliximab, adalimumab and etanercept differ in affinity to the different forms of TNF that exist: soluble TNF (sTNF) and tmTNF.

TNF is synthesized as a membrane-integrated protein, tmTNF. It can be cleaved by metalloprotease TNF-converting enzyme, resulting in sTNF [101]. Infliximab, adalimumab and etanercept are all specific antibodies for sTNF, but differ in binding capacities to tmTNF and their effect on cells expressing tmTNF [74,87,94,102]. Both forms of TNF, tmTNF and sTNF, can activate TNF signaling when binding to TNFRs [103,104]. Two types of TNFR exist: TNFR1 and TNFR2. Soluble TNF preferably binds to TNFR1 whereas tmTNF prefers TNFR2 [47,100]. TNFR1 is constitutively expressed on virtually all nucleated cell types. TNFR2 is highly inducible [105] and its expression is restricted to certain cell types, including T cells [104] and monocytes [106]. Increasing evidence suggests that not only sTNF and TNFR1 but also tmTNF and TNFR2 are involved in the inflammatory response [101,104].

Cellular studies with material derived from sarcoidosis patients are lacking, hence the mechanism of action of anti-TNF biologicals in sarcoidosis is unclear. However, studies using cell cultures derived from patients with Crohn's disease, another granulomatous disease, and from healthy volunteers, give insight into the effect of biologicals against TNF at a cellular level and explain some of the differences in clinical efficacy. Scallon *et al.* [107] showed that tmTNF expressing cells are capable of binding more infliximab per cell than etanercept. It is suggested that the low binding efficiency between tmTNF and etanercept decreases the influence of etanercept on TNF-induced cellular processes [107]. Furthermore, efficient binding between TNF and TNFR might not be completely inhibited. Both infliximab and etanercept are capable of inducing E-selectin expression on human T cells, but only infliximab is able to induce cellular apoptosis [108]. Regarding monocytes, both adalimumab and infliximab induce caspase-dependent apoptosis while etanercept treated cells survive [109].

Moreover, infliximab but not etanercept was able to induce apoptosis and activate caspase 3 in lamina propria cells of patients with Crohn's disease [110]. Etanercept has been studied in patients with Crohn's disease, but was found to be insufficiently effective [47,111].

The ineffectiveness of etanercept treatment in sarcoidosis and in Crohn's disease, both granulomatous diseases, may be caused by the inability of etanercept to induce cellular apoptosis due to incomplete inhibition of tmTNF. Another potential explanation for the difference in effectiveness between agents might be the observation that etanercept and infliximab differently affect expression of certain immune-related genes in human leukocytes (*IFNG, IL1B, IL2Ra*) [112]. Finally, administration routes differ between agents: infliximab is administered intravenously whereas adalimumab and etanercept are given subcutaneously. The latter leads to lower and postponed peak concentrations [113,114] but the clinical significance of the variation in concentration remains unknown.

In conclusion, etanercept was found to be insufficiently effective in granulomatous disease, while infliximab and adalimumab have shown to be of benefit in sarcoidosis patients. This difference may potentially be explained by three factors. First, the lower affinity of etanercept for tmTNF, second, etanercept being unable to induce cellular apoptosis and last, variation in expression of certain immune-related genes.

Adverse effects of anti-TNF biologicals

Besides the beneficial effects of anti-TNF therapeutics, also adverse effects have been reported, that are typical for the targeted inhibitors, the so called biologicals. The most frequently reported side effects in treatment with anti-TNF biologicals are infections [74,87]. In sarcoidosis, most commonly reported adverse effects in infliximab treatment are (upper respiratory tract) infections, coughing and dyspnea [75,76]. In patients treated with adalimumab most frequently reported events were: headache, upper respiratory tract infections and pneumonia, although only a small number of patients has been studied [88]. Serious adverse events that were reported were pneumonia requiring hospitalization and malignancy [75,76,88] (Table 2). From their use in other

treatment indications, we know that anti-TNF drugs might be associated with malignancies [115-117]. In sarcoidosis, seven cases of malignancy have been reported during or after anti-TNF biological treatment [76,98,118-120]. Larger studies are needed to determine whether a causal relation between anti-TNF treatment and malignancy in sarcoidosis exists. The use of anti-TNF biologicals has also been associated with tuberculosis [74,121]. Therefore, all patients should be screened for latent or current tuberculosis infection before treatment initiation. However, the tuberculin skin test that is used most often to screen for latent tuberculosis yields low sensitivity due to T cell anergy in patients with sarcoidosis. The interferon-gamma release assay (IGRA) may be a more accurate method to detect latent tuberculosis, although this suggestion is only based on the results of one small study [122].

Other adverse effects of anti-TNF biologicals are infusion reactions and anaphylactic reactions. Both have been reported for all three anti-TNF biologicals [74,87,94]. Based on research in other IMIDs, it is believed that these reactions are caused by antibodies of isotype IgG towards the specific biological [123]. For sarcoidosis, however, no specific data on the development of antibodies against anti-TNF therapeutics are available. Nevertheless, it is generally believed that the presence of antibodies against infliximab reduces therapy efficacy due to increased infliximab clearance or neutralization of its activity [124-127]. Support for this view may be found in studies in patients with other autoimmune diseases such as rheumatoid arthritis and Crohn's disease, indicating that low- dose methotrexate or another immunosuppressant is preferably added to anti-TNF therapy to reduce antibody-to-antibody formation [128-130]. However, recently it is under debate whether antibody formation against anti-TNF therapy is clinically relevant [131,132]. Nevertheless, further research in the field of antibody development is necessary. So far, clinical implications for sarcoidosis remain unclear.

Table 2. Most common adverse effects of infliximab and adalimumab in sarcoidosis reported in randomized controlled trials.

Agent and adverse effect	Treatment group, frequency (%)	Placebo group, frequency (%)	Reference
Infliximab			
Infections	9/13 (69.2)	3/6 (50.0)	[10]
Upper respiratory tract infection	22/91 (24.2)	8/44 (18.2)	[11]
Coughing	12/91 (13.2)	4/44 (9.1)	[11]
Dyspnea	15/91 (16.5)	5/44 (11.4)	[11]
Pneumonia	2/91 (2.2)	0/44 (0.0)	[11]
Malignancy	2/91 (2.2)	0/44 (0.0)	[11]
Adalimumab			
Headache	7/10 (70.0)	0/6 (0.0)	[16]
Infections (mostly upper respiratory tract infection)	5/10 (50)	3/6 (50)	[16]
Pneumonia	1/10 (10)	0/6 (0.0)	[16]

Another extremely rare and unexpected adverse effect of anti-TNF biologicals is the development of sarcoid-like granulomatosis. Based on one case series (n=10) and several other case reports [133], a causal link between anti-TNF biologicals and the development of granulomatosis in some patients has been suggested [133,134]. The mechanism behind this paradoxical effect remains unknown, but is hypothesized to be related to a lower clearance of the antigen, infection or changes in cytokine environment, all caused by anti-TNF biologicals [134].

Last, the risk of disease relapse after cessation of anti-TNF biologicals remains an important point of discussion. A relapse rate of 74% has been described in patients that were successfully treated with corticosteroids [135]. Cessation of infliximab therapy may also lead to significant relapse and some patients develop symptoms similar to their pre-infliximab treatment status [136]. A study on the incidence of relapse after cessation of infliximab treatment in sarcoidosis in a cohort of 47 patients revealed that 29 patients (62%) relapsed after a mean time of 7.8 months with a mean follow-up time of 36.6 months. In 25% of these patients, relapse occurred within the first four months after treatment discontinuation. Risk factors for relapse were a SUVmax score of the mediastinum ≥ 6.0 on ^{18}F-FDG PET scan or a sIL-2R concentration of ≥ 4000 pg/mL, both at initiation of infliximab therapy, indicating highly active inflammatory disease [137]. These high relapse rates after both corticosteroid and infliximab treatment illustrate the need for parameters that can predict the risk of disease relapse after tapering treatment in sarcoidosis.

In conclusion, apart from their beneficial effects, anti-TNF biologicals have also shown potentially severe side effects, including infections and infusion reactions. Moreover, patients should be closely monitored for possible development of antibodies during treatment and return of disease activity after treatment cessation.

CONCLUSIONS

As a result of the key role of TNF in both the immune response and the formation and maintenance of granulomas in sarcoidosis, inhibiting TNF has become an important target for treatment of the disease, especially when first- and second-line therapeutics have not been sufficiently effective. Non-targeted inhibitors of TNF production, such as thalidomide, pentoxifylline and apremilast, may have some potential in niche indications. Targeted inhibitors such as infliximab and adalimumab, however, are more promising in severe and refractory cases of disease, probably related to their targeted blocking of both sTNF and tmTNF and thereby specific inhibition of the biological activity of TNF. Guidelines on how and when to prescribe these expensive drugs and identification of putative responsive patients are not available yet. Promising tools to predict response are ^{18}F-FDG PET and biomarkers such as sIL-2R. Further research is needed to understand the decisive biological factors in treatment response to anti-TNF therapy and what is the best parameter or combination of parameters to predict treatment-related improvement of disease outcome.

FUTURE PERSPECTIVES

Now that anti-TNF therapy in sarcoidosis has been shown to cause a favorable response in sarcoidosis patients, a number of new issues arise.

The most important topic is the prediction of who will benefit most from this relatively expensive therapy. Theoretically, TNF blocking therapy will likely have most effect in patients with immunologically active disease and evidence of inflammation, because of its target being an important cytokine of the active immune system. So, when no disease activity is detectable and evidence of granulomatous inflammation is available, it may (in our opinion) be unlikely that inhibiting TNF will lead to a substantial clinical benefit.

Subsequently, it is not to be expected that anti-TNF therapy will alleviate symptoms in cases with so-called chronic post-inflammatory fatigue after sarcoidosis [138]. Clinically, *post hoc* analyses by Baughman *et al.* [67,76] and Rossman *et al.* [75] indicate that patients with significant disease severity respond better to therapy than patients with moderate or mild disease severity as measured by FVC. This supports the assumption that anti-TNF therapeutics are of most benefit in more active, severe disease. However, hard evidence to support this assumption is currently lacking.

Nonetheless, in clinical practice it is no sinecure to distinguish active from inactive inflammatory disease and no gold standard on this subject exists. Serum biomarkers have been frequently studied at diagnosis and at different stages of the disease. Both sACE and sIL-2R are known to be informative, however, they need to be further studied to understand the changes in biomarkers during disease development and response to therapy [45,139]. Furthermore, ^{18}F-FDG PET is increasingly applied in the follow-up of sarcoidosis patients. Currently, it is regarded as the most sensitive tool to identify sarcoidosis disease activity [90,140-142]. In addition to the aforementioned study by van Rijswijk *et al.* [79], Keijsers *et al.* [141] also reported a correlation between change of the SUVmax value of the lung parenchyma and the change in VC in twelve patients after 24 weeks of infliximab treatment, indicating that ^{18}F-FDG uptake in sarcoidosis represents active disease [143]. However, large studies on the potential of ^{18}F-FDG PET as a tool to predict a patient's response to infliximab therapy have not been performed [141].

Not only biomarkers may be useful in detection of disease activity and subsequently in prediction of effectiveness of anti-TNF biologicals, also genetics may contribute. Recently, a positive association between response to infliximab and adalimumab in sarcoidosis and the presence of *TNF* -308GG genotype was reported [59]. Remarkably, the same polymorphism was also studied in rheumatoid arthritis and yielded similar results [144,145]. As described before, the *TNF* -308A allele is associated with enhanced production of TNF and variation in phenotypic presentation of the disease [55-59]. Thus, the *TNF* -308 polymorphism potentially plays an important role in the etiology of sarcoidosis. But, again, these results should be interpreted with caution because the *TNF* gene is located closely to and in strong linkage with the *HLA-DRB1* gene [60]. The described association could therefore be caused by the relation between the *HLA-DRB1* gene and suscep-

tibility to sarcoidosis. More research is needed on this matter to assess TNF genotyping utility in predicting response to anti-TNF biologicals. Additionally, research on other genetics variations in association with response to this treatment can be of great value.

In future studies, the use of ^{18}F-FDG PET in the prediction of response to anti-TNF biologicals should be further investigated. Additionally, more data on *TNF* G-308A and *HLA-DRB1* could enlighten whether these genes in fact are of clinical use in predicting response to anti-TNF biologicals.

Another problem encountered in predicting individual response to anti-TNF therapy is the lack of a universal end-point in sarcoidosis treatment. Due to the many different phenotypes and degrees of severity of the disease, numerous different parameters have been used to describe response to therapy. In several studies, discrepancy between objective and subjective parameters is present [143]. For instance, up to 70% of patients experience fatigue also after disease biomarkers such as sIL-2R and sACE have returned to physiological, normal levels after anti-TNF treatment [146]. In addition, lung function parameters may not improve upon initiation of therapy when tissue is dysfunctional due to persistent fibrosis and not due to an inflammatory component. Therefore, therapeutic effectiveness in sarcoidosis patients is hard to evaluate. In future, intervention studies should include not only evaluation from a clinical point of view, but also comprise patient reported outcomes such as quality of life [147].

Finally, a new anti-TNF biological was registered: golimumab. Golimumab has high affinity for both sTNF and tmTNF [102]. So far, this biological is available for treatment in rheumatoid arthritis and psoriatic arthritis. Recently, a study in chronic sarcoidosis patients with pulmonary and/or cutaneous involvement has been conducted (clinicaltrials.gov trial number NCT00955279). Even though study results are to be awaited, golimumab might be added to the range of anti-TNF biologicals for sarcoidosis in the near future.

In conclusion, prediction of patients' response to anti-TNF biologicals remains a challenge at this moment. Future studies should shed light on the potential role of ^{18}F-FDG PET in predicting response to anti-TNF biological treatment and also on finding universal parameters to evaluate therapy response accurately in all sarcoidosis patients.

REFERENCES

1. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med* 2007; 357: 2153-2165.
2. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
3. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. *Lancet* 2003; 361: 1111-1118.
4. Dastoori M, Fedele S, Leao JC, Porter SR. Sarcoidosis - a clinically orientated review. *J Oral Pathol Med* 2013; 42: 281-289.
5. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager Jr H, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R, Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med* 2001; 164: 1885-1889.
6. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. *Am J Respir Crit Care Med* 2011; 183: 573-581.
7. Rao DA, Dellaripa PF. Extrapulmonary manifestations of sarcoidosis. *Rheum Dis Clin North Am* 2013; 39: 277-297.
8. Israel-Biet D, Valeyre D. Diagnosis of pulmonary sarcoidosis. *Curr Opin Pulm Med* 2013; 19: 510-515.
9. Heinle R, Chang C. Diagnostic criteria for sarcoidosis. *Autoimmun Rev* 2014; 13: 383-387.
10. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. *Lancet* 2013; 383: 1155-1167.
11. Hillerdal G, Nou E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. *Am Rev Respir Dis* 1984; 130: 29-32.
12. Morimoto T, Azuma A, Abe S, Usuki J, Kudoh S, Sugisaki K, Oritsu M, Nukiwa T. Epidemiology of sarcoidosis in Japan. *Eur Respir J* 2008; 31: 372-379.
13. Judson MA, Boan AD, Lackland DT. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. *Sarcoidosis Vasc Diffuse Lung Dis* 2012; 29: 119-127.
14. Judson MA, Thompson BW, Rabin DL, Steimel J, Knattereud GL, Lackland DT, Rose C, Rand CS, Baughman RP, Teirstein AS, ACCESS Research Group. The diagnostic pathway to sarcoidosis. *Chest* 2003; 123: 406-412.
15. Grunewald J. Genetics of sarcoidosis. *Curr Opin Pulm Med* 2008; 14: 434-439.
16. Cozier YC, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: data from the Black Women's Health Study. *Chest* 2011; 139: 144-150.
17. Rybicki BA, Major M, Popovich Jr J, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. *Am J Epidemiol* 1997; 145: 234-241.
18. James DG, Neville E, Siltzbach LE. A worldwide review of sarcoidosis. *Ann N Y Acad Sci* 1976; 278: 321-334.
19. Pietinalho A, Ohmichi M, Hiraga Y, Lofroos AB, Selroos O. The mode of presentation of sarcoidosis in Finland and Hokkaido, Japan. A comparative analysis of 571 Finnish and 686 Japanese patients. *Sarcoidosis Vasc Diffuse Lung Dis* 1996; 13: 159-166.
20. Gideon NM, Mannino DM. Sarcoidosis mortality in the United States 1979-1991: an analysis of multiple-cause mortality data. *Am J Med* 1996; 100: 423-427.
21. Swigris JJ, Olson AL, Huie TJ, Fernandez-Perez ER, Solomon J, Sprunger D, Brown KK. Sarcoidosis-related mortality in the United States from 1988 to 2007. *Am J Respir Crit Care Med* 2011; 183: 1524-1530.
22. Iwai K, Tachibana T, Takemura T, Matsui Y, Kitaichi M, Kawabata Y. Pathological studies on sarcoidosis autopsy. I. Epidemiological features of 320 cases in Japan. *Acta Pathol Jpn* 1993; 43: 372-376.
23. Iwai K, Sekiguti M, Hosoda Y, DeRemee RA, Tazelaar HD, Sharma OP, Maheshwari A, Noguchi TI. Racial difference in cardiac sarcoidosis incidence observed at autopsy. *Sarcoidosis* 1994; 11: 26-31.
24. Siltzbach LE, James DG, Neville E, Turiaf J, Battesti JP, Sharma OP, Hosoda Y, Mikami R, Odaka M. Course and prognosis of sarcoidosis around the world. *Am J Med* 1974; 57: 847-852.

25. Prasse A, Georges CG, Biller H, Hamm H, Matthys H, Luttmann W, Virchow Jr JC. Th1 cytokine pattern in sarcoidosis is expressed by bronchoalveolar CD4+ and CD8+ T cells. *Clin Exp Immunol* 2000; 122: 241-248.
26. Baughman RP, Strohofer SA, Buchsbaum J, Lower EE. Release of tumor necrosis factor by alveolar macrophages of patients with sarcoidosis. *J Lab Clin Med* 1990; 115: 36-42.
27. Pantelidis P, McGrath DS, Southcott AM, du Bois RM. Single-cell analysis: a novel approach to tumour necrosis factor-alpha synthesis and secretion in sarcoidosis. *Eur Respir J* 2002; 20: 1179-1184.
28. Dai H, Guzman J, Chen B, Costabel U. Production of soluble tumor necrosis factor receptors and tumor necrosis factor-alpha by alveolar macrophages in sarcoidosis and extrinsic allergic alveolitis. *Chest* 2005; 127: 251-256.
29. Zissel G, Prasse A, Muller-Quernheim J. Immunologic response of sarcoidosis. *Semin Respir Crit Care Med* 2010; 31: 390-403.
30. Broos CE, van Nimwegen M, Hoogsteden HC, Hendriks RW, Kool M, van den Blink B. Granuloma Formation in Pulmonary Sarcoidosis. *Front Immunol* 2013; 4: 437.
31. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* 2011; 305: 391-399.
32. Lugo-Villarino G, Hudrisier D, Benard A, Neyrolles O. Emerging trends in the formation and function of tuberculosis granulomas. *Front Immunol* 2012; 3: 405.
33. Kaufmann SH. Protection against tuberculosis: cytokines, T cells, and macrophages. *Ann Rheum Dis* 2002; 61 Suppl 2: ii54-8.
34. Reich JM. On the nature of sarcoidosis. *Eur J Intern Med* 2012; 23: 105-109.
35. Iannuzzi MC. Genetics of sarcoidosis. *Semin Respir Crit Care Med* 2007; 28: 15-21.
36. Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, Terrin ML, Moller DR, Barnard J, Baughman RP, DePalo L, Hunninghake G, Johns C, Judson MA, Knatterud GL, McLennan G, Newman LS, Rabin DL, Rose C, Teirstein AS, Weinberger SE, Yeager H, Cherniack R, ACCESS Research Group. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). *Am J Respir Crit Care Med* 2001; 164: 2085-2091.
37. van Moorsel CH, Christiani DC. Genetic susceptibility to sarcoidosis, a chronic inflammatory disorder. *Am J Respir Crit Care Med* 2012; 186: 816-818.
38. Muller-Quernheim J, Prasse A, Zissel G. Pathogenesis of sarcoidosis. *Presse Med* 2012; 41: e275-87.
39. Drent M, Cremers JP, Jansen TL. Pulmonology meets rheumatology in sarcoidosis: a review on the therapeutic approach. *Curr Opin Rheumatol* 2014; 26: 276-284.
40. Paramothayan S, Lasserson T. Treatments for pulmonary sarcoidosis. *Respir Med* 2008; 102: 1-9.
41. Grutters JC, van den Bosch JM. Corticosteroid treatment in sarcoidosis. *Eur Respir J* 2006; 28: 627-636.
42. Paramothayan NS, Lasserson TJ, Jones PW. Corticosteroids for pulmonary sarcoidosis. *Cochrane Database Syst Rev* 2005; (2): CD001114.
43. Pietinalho A, Tukiainen P, Haahtela T, Persson T, Selroos O, Finnish Pulmonary Sarcoidosis Study Group. Early treatment of stage II sarcoidosis improves 5-year pulmonary function. *Chest* 2002; 121: 24-31.
44. Vorselaars AD, van Moorsel CH, Deneer VH, Grutters JC. Current therapy in sarcoidosis, the role of existing drugs and future medicine. *Inflamm Allergy Drug Targets* 2013; 12: 369-377.
45. Vorselaars AD, Wuyts WA, Vorselaars VM, Zanen P, Deneer VH, Veltkamp M, Thomeer M, van Moorsel CH, Grutters JC. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* 2013; 144: 805-812.
46. Feldmann M. Development of anti-TNF therapy for rheumatoid arthritis. *Nat Rev Immunol* 2002; 2: 364-371.
47. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008; 117: 244-279.
48. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996; 334: 1717-1725.
49. Kasahara K, Kobayashi K, Shikama Y, Yoneya I, Kaga S, Hashimoto M, Odagiri T, Soejima K, Ide H, Takahashi T. The role of monokines in granuloma formation in mice: the ability of interleukin 1 and tumor necrosis factor-alpha to induce lung granulomas. *Clin Immunol Immunopathol* 1989; 51: 419-425.

50. Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y, Richards E, Jungbluth A, Wada H, Moore M, Williamson B, Basu S, Old LJ. Characterization of tumor necrosis factor-deficient mice. *Proc Natl Acad Sci U S A* 1997; 94: 8093-8098.
51. Roach DR, Bean AG, Demangel C, France MP, Briscoe H, Britton WJ. TNF regulates chemokine induction essential for cell recruitment, granuloma formation, and clearance of mycobacterial infection. *J Immunol* 2002; 168: 4620-4627.
52. Ziegenhagen MW, Benner UK, Zissel G, Zabel P, Schlaak M, Muller-Quernheim J. Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am J Respir Crit Care Med* 1997; 156: 1586-1592.
53. Braam AW, de Haan SN, Vorselaars AD, Rijkers GT, Grutters JC, van den Elshout FJ, Korenromp IH. Influence of repeated maximal exercise testing on biomarkers and fatigue in sarcoidosis. *Brain Behav Immun* 2013; 33: 57-64.
54. Baydur A, Alavy B, Nawathe A, Liu S, Louie S, Sharma OP. Fatigue and plasma cytokine concentrations at rest and during exercise in patients with sarcoidosis. *Clin Respir J* 2011; 5: 156-164.
55. Song GG, Kim JH, Lee YH. Associations between TNF-alpha -308 A/G and lymphotoxin-alpha +252 A/G polymorphisms and susceptibility to sarcoidosis: a meta-analysis. *Mol Biol Rep* 2014; 41: 259-267.
56. Wijnen PA, Nelemans PJ, Verschakelen JA, Bekers O, Voorter CE, Drent M. The role of tumor necrosis factor alpha G-308A polymorphisms in the course of pulmonary sarcoidosis. *Tissue Antigens* 2010; 75: 262-268.
57. Grutters JC, Sato H, Pantelidis P, Lagan AL, McGrath DS, Lammers JW, van den Bosch JM, Wells AU, du Bois RM, Welsh KI. Increased frequency of the uncommon tumor necrosis factor -857T allele in British and Dutch patients with sarcoidosis. *Am J Respir Crit Care Med* 2002; 165: 1119-1124.
58. Medica I, Kastrin A, Maver A, Peterlin B. Role of genetic polymorphisms in ACE and TNF-alpha gene in sarcoidosis: a meta-analysis. *J Hum Genet* 2007; 52: 836-847.
59. Wijnen PA, Cremers JP, Nelemans PJ, Erckens RJ, Hoitsma E, Jansen TL, Bekers O, Drent M. Association of the TNF-alpha G-308A polymorphism with TNF-inhibitor response in sarcoidosis. *Eur Respir J* 2014; 43: 1730-1739.
60. Grunewald J, Eklund A, Olerup O. Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients. *Am J Respir Crit Care Med* 2004; 169: 696-702.
61. Tavares JL, Wangoo A, Dilworth P, Marshall B, Kotecha S, Shaw RJ. Thalidomide reduces tumour necrosis factor-alpha production by human alveolar macrophages. *Respir Med* 1997; 91: 31-39.
62. Ye Q, Chen B, Tong Z, Nakamura S, Sarria R, Costabel U, Guzman J. Thalidomide reduces IL-18, IL-8 and TNF-alpha release from alveolar macrophages in interstitial lung disease. *Eur Respir J* 2006; 28: 824-831.
63. Fazzi P, Manni E, Cristofani R, Cei G, Piazza S, Calabrese R, Antonelli A, Siciliano G, Barachini P, Carpi A. Thalidomide for improving cutaneous and pulmonary sarcoidosis in patients resistant or with contraindications to corticosteroids. *Biomed Pharmacother* 2012; 66: 300-307.
64. Baughman RP, Judson MA, Teirstein AS, Moller DR, Lower EE. Thalidomide for chronic sarcoidosis. *Chest* 2002; 122: 227-232.
65. Nguyen YT, Dupuy A, Cordoliani F, Vignon-Pennamen MD, Lebbe C, Morel P, Rybojad M. Treatment of cutaneous sarcoidosis with thalidomide. *J Am Acad Dermatol* 2004; 50: 235-241.
66. Judson MA, Silvestri J, Hartung C, Byars T, Cox CE. The effect of thalidomide on corticosteroid-dependent pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006; 23: 51-57.
67. Baughman RP, Costabel U, du Bois RM. Treatment of sarcoidosis. *Clin Chest Med* 2008; 29: 533-548.
68. Marques LJ, Zheng L, Poulakis N, Guzman J, Costabel U. Pentoxifylline inhibits TNF-alpha production from human alveolar macrophages. *Am J Respir Crit Care Med* 1999; 159: 508-511.
69. Tong Z, Dai H, Chen B, Abdoh Z, Guzman J, Costabel U. Inhibition of cytokine release from alveolar macrophages in pulmonary sarcoidosis by pentoxifylline: comparison with dexamethasone. *Chest* 2003; 124: 1526-1532.
70. Katakami Y, Nakao Y, Koizumi T, Katakami N, Ogawa R, Fujita T. Regulation of tumour necrosis factor production by mouse peritoneal macrophages: the role of cellular cyclic AMP. *Immunology* 1988; 64: 719-724.
71. Zabel P, Entzian P, Dalhoff K, Schlaak M. Pentoxifylline in treatment of sarcoidosis. *Am J Respir Crit Care Med* 1997; 155: 1665-1669.
72. Park MK, Fontana J, Babaali H, Gilbert-McClain LI, Stylianou M, Joo J, Moss J, Manganiello VC. Steroid-sparing effects

of pentoxifylline in pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2009; 26: 121-131.

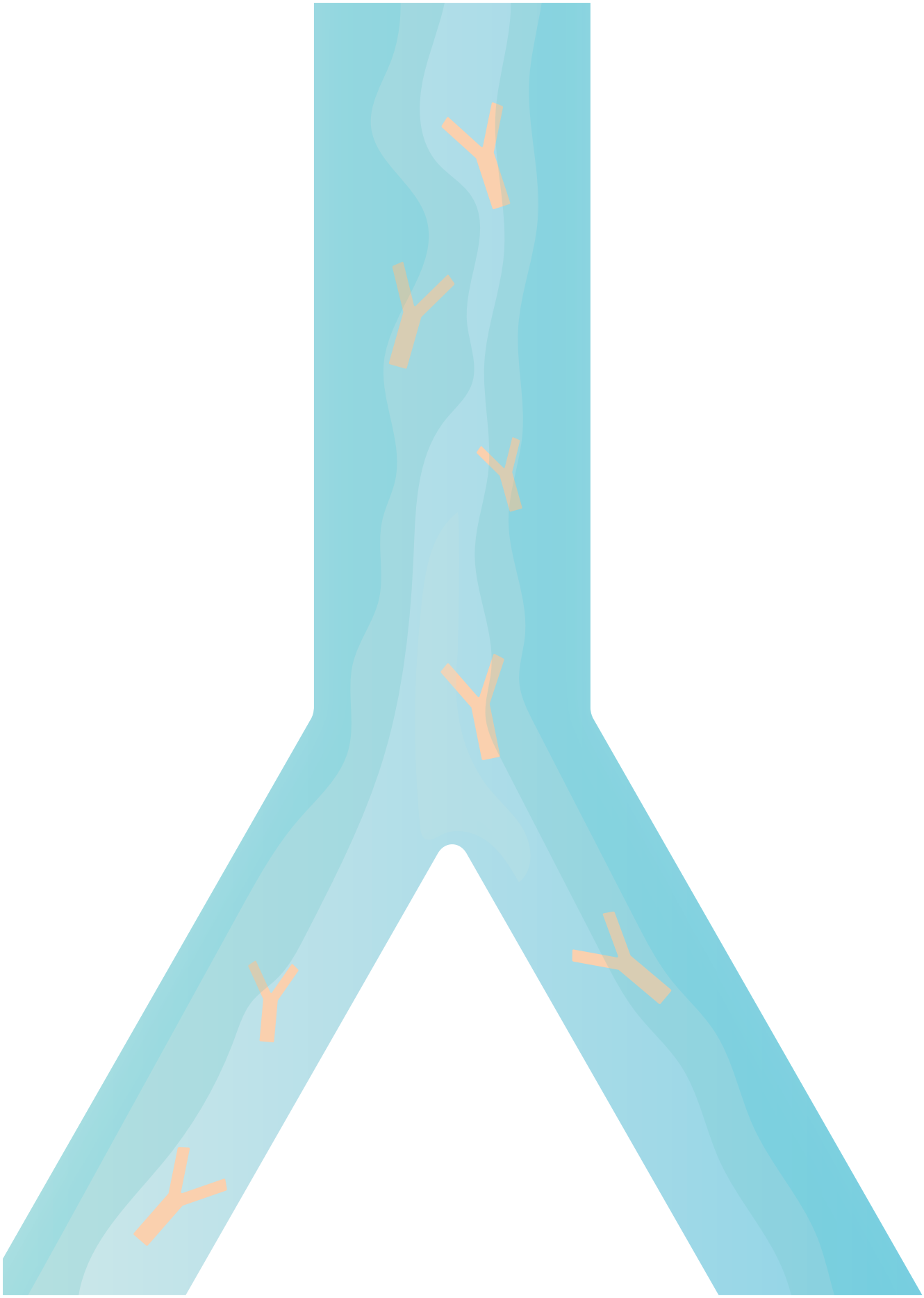
73. Baughman RP, Judson MA, Ingledue R, Craft NL, Lower EE. Efficacy and safety of apremilast in chronic cutaneous sarcoidosis. *Arch Dermatol* 2012; 148: 262-264.
74. Centocor. Remicade(R) (infliximab), prescribing information. 2006.
75. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller Jr W, Sands BE. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006; 23: 201-208.
76. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
77. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, Drent M, Centocor T48 Sarcoidosis Investigators. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J* 2008; 31: 1189-1196.
78. Judson MA, Baughman RP, Costabel U, Mack M, Barnathan ES. The potential additional benefit of infliximab in patients with chronic pulmonary sarcoidosis already receiving corticosteroids: A retrospective analysis from a randomized clinical trial. *Respir Med* 2014; 108: 189-194.
79. van Rijswijk HNAJ, Vorselaars ADM, Ruven HTJ, Keijsers RGM, Zanen P, Korenromp IHE, Grutters JC. Changes in disease activity, lung function and quality of life in patients with refractory sarcoidosis after anti-TNF treatment. *Expert Opinion on Orphan Drugs* 2013; 1: 437-443.
80. Hostettler KE, Studler U, Tamm M, Brutsche MH. Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218-224.
81. Russell E, Luk F, Manocha S, Ho T, O'Connor C, Hussain H. Long term follow-up of infliximab efficacy in pulmonary and extra-pulmonary sarcoidosis refractory to conventional therapy. *Semin Arthritis Rheum* 2013; 43: 119-124.
82. Theodossiadis PG, Markomichelakis NN, Sfikakis PP. Tumor necrosis factor antagonists: preliminary evidence for an emerging approach in the treatment of ocular inflammation. *Retina* 2007; 27: 399-413.
83. Barnabe C, McMeekin J, Howarth A, Martin L. Successful treatment of cardiac sarcoidosis with infliximab. *J Rheumatol* 2008; 35: 1686-1687.
84. Santos E, Shaunak S, Renowden S, Scolding NJ. Treatment of refractory neurosarcoidosis with Infliximab. *J Neurol Neurosurg Psychiatry* 2010; 81: 241-246.
85. Pereira J, Anderson NE, McAuley D, Bergin P, Kilfoyle D, Fink J. Medically refractory neurosarcoidosis treated with infliximab. *Intern Med J* 2011; 41: 354-357.
86. Hasni SA, Kunz D, Finzel K, Gruber BL. Osseous sarcoidosis treated with tumor necrosis factor-inhibitors: case report and review of the literature. *Spine (Phila Pa 1976)* 2010; 35: E904-7.
87. Abbott Laboratories. Humira(R) (adalimumab), prescribing information. 2007.
88. Pariser RJ, Paul J, Hirano S, Torosky C, Smith M. A double-blind, randomized, placebo-controlled trial of adalimumab in the treatment of cutaneous sarcoidosis. *J Am Acad Dermatol* 2013; 68: 765-773.
89. Erckens RJ, Mostard RL, Wijnen PA, Schouten JS, Drent M. Adalimumab successful in sarcoidosis patients with refractory chronic non-infectious uveitis. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 713-720.
90. Milman N, Graudal N, Loft A, Mortensen J, Larsen J, Baslund B. Effect of the TNF-alpha inhibitor adalimumab in patients with recalcitrant sarcoidosis: a prospective observational study using FDG-PET. *Clin Respir J* 2012; 6: 238-247.
91. Sweiss NJ, Noth I, Mirsaeidi M, Zhang W, Naureckas ET, Hogarth DK, Strek M, Caligiuri P, Machado RF, Niewold TB, Garcia JG, Pangan AL, Baughman RP. Efficacy results of a 52-week trial of adalimumab in the treatment of refractory sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 46-54.
92. Patel SR. Systemic sarcoidosis with bone marrow involvement responding to therapy with adalimumab: a case report. *J Med Case Rep* 2009; 3: 8573.
93. Diaz-Llopis M, Garcia-Delpech S, Salom D, Udaondo P, Hernandez-Garfella M, Bosch-Morell F, Quijada A, Romero FJ. Adalimumab therapy for refractory uveitis: a pilot study. *J Ocul Pharmacol Ther* 2008; 24: 351-361.

94. Immunex Corp. Enbrel(R) (etanercept), precribing information. 2007.
95. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999; 354: 1932-1939.
96. Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999; 340: 253-259.
97. Sandborn WJ, Hanauer SB, Katz S, Safdi M, Wolf DG, Baerg RD, Tremaine WJ, Johnson T, Diehl NN, Zinsmeister AR. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2001; 121: 1088-1094.
98. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest* 2003; 124: 177-185.
99. Baughman RP, Lower EE, Bradley DA, Raymond LA, Kaufman A. Etanercept for refractory ocular sarcoidosis: results of a double-blind randomized trial. *Chest* 2005; 128: 1062-1047.
100. Silva LC, Ortigosa LC, Benard G. Anti-TNF-alpha agents in the treatment of immune-mediated inflammatory diseases: mechanisms of action and pitfalls. *Immunotherapy* 2010; 2: 817-833.
101. Horiuchi T, Mitoma H, Harashima S, Tsukamoto H, Shimoda T. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. *Rheumatology (Oxford)* 2010; 49: 1215-1228.
102. Taylor PC. Pharmacology of TNF blockade in rheumatoid arthritis and other chronic inflammatory diseases. *Curr Opin Pharmacol* 2010; 10: 308-315.
103. Ruspi G, Schmidt EM, McCann F, Feldmann M, Williams RO, Stoop AA, Dean JL. TNFR2 increases the sensitivity of ligand-induced activation of the p38 MAPK and NF-kappaB pathways and signals TRAF2 protein degradation in macrophages. *Cell Signal* 2013; 26: 683-690.
104. Faustman D, Davis M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov* 2010; 9: 482-493.
105. Vandenabeele P, Declercq W, Beyaert R, Fiers W. Two tumour necrosis factor receptors: structure and function. *Trends Cell Biol* 1995; 5: 392-399.
106. Hijdra D, Vorselaars AD, Grutters JC, Claessen AM, Rijkers GT. Differential expression of TNFR1 (CD120a) and TNFR2 (CD120b) on subpopulations of human monocytes. *J Inflamm (Lond)* 2012; 9: 38-9255-9-38.
107. Scallon B, Cai A, Solowski N, Rosenberg A, Song XY, Shealy D, Wagner C. Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther* 2002; 301: 418-426.
108. Mitoma H, Horiuchi T, Hatta N, Tsukamoto H, Harashima S, Kikuchi Y, Otsuka J, Okamura S, Fujita S, Harada M. Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-alpha. *Gastroenterology* 2005; 128: 376-392.
109. Shen C, Assche GV, Colpaert S, Maerten P, Geboes K, Rutgeerts P, Ceuppens JL. Adalimumab induces apoptosis of human monocytes: a comparative study with infliximab and etanercept. *Aliment Pharmacol Ther* 2005; 21: 251-258.
110. Van den Brande JM, Braat H, van den Brink GR, Versteeg HH, Bauer CA, Hoedemaeker I, van Montfrans C, Hommes DW, Peppelenbosch MP, van Deventer SJ. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology* 2003; 124: 1774-1785.
111. Sandborn WJ. Highlights in Anti-Tumor Necrosis Factor Monitoring. *Gastroenterology & Hepatology* 2012; 8: 1-12.
112. Haider AS, Cardinale IR, Whynot JA, Krueger JG. Effects of etanercept are distinct from infliximab in modulating proinflammatory genes in activated human leukocytes. *J Investig Dermatol Symp Proc* 2007; 12: 9-15.
113. Nestorov I. Clinical pharmacokinetics of tumor necrosis factor antagonists. *J Rheumatol Suppl* 2005; 74: 13-18.
114. Nestorov I. Clinical pharmacokinetics of TNF antagonists: how do they differ? *Semin Arthritis Rheum* 2005; 34: 12-18.
115. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis

and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 2006; 295: 2275-2285.

116. Fellermann K. Adverse events of tumor necrosis factor inhibitors. *Dig Dis* 2013; 31: 374-378.
117. Pallavicini FB, Caporali R, Sarzi-Puttini P, Atzeni F, Bazzani C, Gorla R, Marchesoni A, Favalli EG, Montecucco C. Tumour necrosis factor antagonist therapy and cancer development: analysis of the LORHEN registry. *Autoimmun Rev* 2010; 9: 175-180.
118. Doty JD, Mazur JE, Judson MA. Treatment of sarcoidosis with infliximab. *Chest* 2005; 127: 1064-1071.
119. Pritchard C, Nadarajah K. Tumour necrosis factor alpha inhibitor treatment for sarcoidosis refractory to conventional treatments: a report of five patients. *Ann Rheum Dis* 2004; 63: 318-320.
120. Vorselaars AD, Sjogren EV, van Moorsel CH, Grutters JC. Bilateral Vocal Cord Carcinoma in a Sarcoidosis Patient during Infliximab Therapy. *Case Rep Pulmonol* 2013; 2013: 308092.
121. Cantini F, Niccoli L, Goletti D. Adalimumab, etanercept, infliximab, and the risk of tuberculosis: data from clinical trials, national registries, and postmarketing surveillance. *J Rheumatol Suppl* 2014; 91:47-55.
122. Gupta D, Kumar S, Aggarwal AN, Verma I, Agarwal R. Interferon gamma release assay (QuantiFERON-TB Gold In Tube) in patients of sarcoidosis from a population with high prevalence of tuberculosis infection. *Sarcoidosis Vasc Diffuse Lung Dis* 2011; 28: 95-101.
123. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 34: 51-58.
124. Baert F, Noman M, Vermeire S, Van Assche G, D'Haens G, Carbonez A, Rutgeerts P. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003; 348: 601-608.
125. Farrell RJ, Alsahli M, Jeen YT, Falchuk KR, Peppercorn MA, Michetti P. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn's disease: a randomized controlled trial. *Gastroenterology* 2003; 124: 917-924.
126. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2008; 84: 548-558.
127. Xu Z, Seitz K, Fasanmade A, Ford J, Williamson P, Xu W, Davis HM, Zhou H. Population pharmacokinetics of infliximab in patients with ankylosing spondylitis. *J Clin Pharmacol* 2008; 48: 681-695.
128. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 1552-1563.
129. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P, ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; 359: 1541-1549.
130. Miele E, Markowitz JE, Mamula P, Baldassano RN. Human antichimeric antibody in children and young adults with inflammatory bowel disease receiving infliximab. *J Pediatr Gastroenterol Nutr* 2004; 38: 502-508.
131. Feagan BG, McDonald JW, Panaccione R, Enns RA, Bernstein CN, Ponich TP, Bourdages R, Macintosh DG, Dallaire C, Cohen A, Fedorak RN, Pare P, Bitton A, Saibil F, Anderson F, Donner A, Wong CJ, Zou G, Vandervoort MK, Hopkins M, Greenberg GR. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. *Gastroenterology* 2014; 146: 681-688.
132. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. *Ann Rheum Dis* 2012; 71: 1914-1915.
133. Tong D, Manolios N, Howe G, Spencer D. New onset sarcoid-like granulomatosis developing during anti-TNF therapy: an under-recognised complication. *Intern Med J* 2012; 42: 89-94.
134. Daien CI, Monnier A, Claudepierre P, Constantin A, Eschard JP, Houvenagel E, Samimi M, Pavy S, Pertuiset E, Toussirot E, Combe B, Morel J, Club Rhumatismes et Inflammation (CRI). Sarcoid-like granulomatosis in patients treated with tumor necrosis factor blockers: 10 cases. *Rheumatology (Oxford)* 2009; 48: 883-886.

135. Gottlieb JE, Israel HL, Steiner RM, Triolo J, Patrick H. Outcome in sarcoidosis. The relationship of relapse to corticosteroid therapy. *Chest* 1997; 111: 623-631.
136. Panselinas E, Rodgers JK, Judson MA. Clinical outcomes in sarcoidosis after cessation of infliximab treatment. *Respirology* 2009; 14: 522-528.
137. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijsers RG, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J* 2014; 43: 602-609.
138. Korenromp IH, Heijnen CJ, Vogels OJ, van den Bosch JM, Grutters JC. Characterization of chronic fatigue in patients with sarcoidosis in clinical remission. *Chest* 2011; 140: 441-447.
139. Rothkrantz-Kos S, van Dieijen-Visser MP, Mulder PG, Drent M. Potential usefulness of inflammatory markers to monitor respiratory functional impairment in sarcoidosis. *Clin Chem* 2003; 49: 1510-1517.
140. Keijsers RG, Verzijlbergen JF, van Diepen DM, van den Bosch JM, Grutters JC. 18F-FDG PET in sarcoidosis: an observational study in 12 patients treated with infliximab. *Sarcoidosis Vasc Diffuse Lung Dis* 2008; 25: 143-149.
141. Keijsers RG, van den Heuvel DA, Grutters JC. Imaging the inflammatory activity of sarcoidosis. *Eur Respir J* 2013; 41: 743-751.
142. Mostard RL, van Kroonenburgh MJ, Drent M. The role of the PET scan in the management of sarcoidosis. *Curr Opin Pulm Med* 2013; 19: 538-544.
143. Keijsers RG, Grutters JC, Thomeer M, Du Bois RM, Van Buul MM, Lavalaye J, Van Den Bosch JM, Verzijlbergen FJ. Imaging the inflammatory activity of sarcoidosis: sensitivity and inter observer agreement of (67)Ga imaging and (18)F-FDG PET. *Q J Nucl Med Mol Imaging* 2011; 55: 66-71.
144. Marotte H, Miossec P. Biomarkers for prediction of TNFalpha blockers response in rheumatoid arthritis. *Joint Bone Spine* 2010; 77: 297-305.
145. Mugnier B, Balandraud N, Darque A, Roudier C, Roudier J, Reviron D. Polymorphism at position -308 of the tumor necrosis factor alpha gene influences outcome of infliximab therapy in rheumatoid arthritis. *Arthritis Rheum* 2003; 48: 1849-1852.
146. De Vries J, Rothkrantz-Kos S, van Dieijen-Visser MP, Drent M. The relationship between fatigue and clinical parameters in pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2004; 21: 127-136.
147. Baughman RP, Drent M, Culver DA, Grutters JC, Handa T, Humbert M, Judson MA, Lower EE, Mana J, Pereira CA, Prasse A, Sulica R, Valyere D, Vucinic V, Wells AU. Endpoints for clinical trials of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2012; 29: 90-98.



3

Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis

Heleen A Crommelin*
Adriane DM Vorselaars*
Vera HM Deneer
Bob Meek
Anke ME Claessen
Ruth GM Keijsers
Coline HM van Moorsel
Jan C Grutters

**Authors contributed equally to the manuscript and therefore share first authorship.*

Eur Respir J. 2015 Jul;46(1):175-185.

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 ^{18}F-fluorodeoxyglucose (^{18}F-FDG) by positron emission tomography (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 in the six months before start, lung function decreased. Maximum standardized uptake value (SUVmax) of pulmonary parenchyma on ^{18}F-FDG PET scan decreased by 3.93 (p<0.0001). High SUVmax of pulmonary parenchyma at baseline predicted FVC improvement (R=0.62, p=0.0004). An overall beneficial response was seen in 79% of patients and partial response in 17% of patients. 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 ^{18}F-FDG PET positive 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 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 forced vital capacity (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 ^{18}F-fluorodeoxyglucose (^{18}F-FDG) by positron emission tomography (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 against infliximab can result in decreased levels of infliximab and

diminished drug efficacy. Additionally, antibodies against infliximab can sometimes cause allergic reactions [12,24-26]. It is not known whether formation of antibodies against infliximab and associated low trough levels play a role in the 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 against infliximab are related to the initial response rate after 26 weeks.

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 center 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 American Thoracic Society/European Respiratory Society criteria [1]. The treating physician judged disease severity at the moment of initiation based on loss of function (*e.g.* lung function or cardiac function), impaired quality of life and disease activity on ^{18}F-FDG PET. Exclusion criteria were vaccination with live or bacterial vaccines 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 of St Antonius Hospital Nieuwegein (registration number LTME/R-10.13A, acronym INFLIXIMAB) 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 FVC, forced expiratory volume in one second (FEV1) and diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (DLCOc)). Small fiber neurop-

athy was tested using the small fiber neuropathy screening list (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 % predicted and percentage of patients with an increase of ≥ 5% predicted and ≥ 10% predicted [28].

Inflammatory response

Parameters in the inflammation category included the biomarkers soluble interleukin-2 receptor (sIL-2R), angiotensin-converting enzyme (ACE) and ^{18}F-FDG PET maximum standardized 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 (ROIs) were drawn over the visually affected part of the organ to measure the SUVmax. ROI was drawn at the same lesion/area at baseline and follow-up scan. ROI drawing was performed using the automatic ROI drawing tool in the Hermes Diagnostics program (Hermes Medical Solutions, Stockholm, Sweden). Blood glucose level was measured before injecting FDG in all patients. FDG was administered when the plasma glucose level was < 7 mmol/L.

Quality of life

Finally, quality of life was measured using 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 (SF)-36. An improvement of 10 points was considered clinically relevant.

Composite overall response

In addition we reported the response rate as a composite of three categories: organ function, inflammation and quality of life. Improvement in a category was scored only when one of the parameters improved significantly without deterioration of the others. For the functional response in pulmonary sarcoidosis we used an improvement in FVC of ≥ 5% predicted. A decrease in biomarkers or SUVmax > 40% of baseline was considered a relevant improvement. Changes within the normal range of sIL-2R, ACE and SUVmax on ^{18}F-FDG PET scan were not taken into account when gauging response. Change in SUVmax of the target organ was regarded as superior to change in inflammatory biomarkers. Clinically relevant improvement in two or three categories was considered good or excellent response, in one category as partial response and in none of the categories as nonresponse.

Infliximab trough levels and antibodies against infliximab

Infliximab trough levels were measured using an ELISA (Sanquin, The Netherlands) [29]. This ELISA only detects infliximab that is able to bind TNF. It does not detect immune complexes consisting

of infliximab and TNF or infliximab bound to neutralizing antibodies against infliximab. The presence of antibodies against infliximab 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; IBM, Armonk, NY, USA). Graphs were created using GraphPad Prism 5.0. $p < 0.05$ 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 (Figure 1). 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 fiber 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).

Organ function

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

Notably, even in patients with an extrapulmonary treatment indication, mean FVC and FEV1 increased significantly, by 3.88% predicted (p=0.027) and 3.54% predicted (p=0.034), respectively. Moreover, an increase of ≥ 5% predicted was seen in 37% of these patients for each of these parameters (Figure 2A).

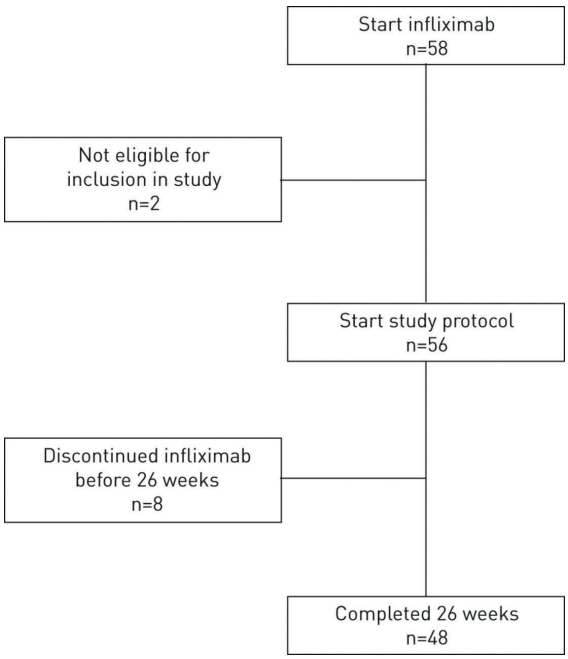


Figure 1. Flowchart of patient inclusion.
Two patients were excluded from the study protocol, one due to incapability to give informed consent based on mental retardation and one because his cold agglutinin disease would not permit routine sampling at every visit.

Table 1. Baseline characteristics of study subjects.

Subjects, n	56
Male	36 (64.3)
Caucasian	49 (87.5)
Age at initiation of infliximab therapy, years	48.7 ± 10.1
Disease duration at initiation of infliximab therapy, years	6.8 ± 7.1
Diagnosis of sarcoidosis	
Biopsy	52 (92.9)
Bronchoalveolar lavage	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	
0	5 (8.9)
I	6 (10.7)
II	16 (28.6)
III	14 (25.0)
IV	15 (26.8)

Table 1. Continued

Main treatment indication	
Pulmonary	34 (60.7)
Cardiac	2 (3.6)
Small fiber 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	52 (92.9)
Medication use prior to initiation of infliximab	
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	52 (92.2)
Concomitant medication	
Corticosteroids	24 (42.9)
Methotrexate	46 (82.1)
Azathioprine	4 (7.1)
Leflunomide	1 (1.8)
None	0 (0)
Pulmonary function parameters	
FVC, L (% predicted)	3.32 (78.8)
FEV1, L (% predicted)	2.30 (66.8)
DLCOc, L (% predicted)	6.01 (59.8)
6-MWD, 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
ACE, U/L	89.7 ± 49.7
ACE Z-score	4.3 ± 4.8
sIL-2R, pg/mL	8824 ± 8503

Data are presented as n (%) or mean ± SD unless otherwise stated. TNF: tumor necrosis factor; FVC: forced vital capacity, FEV1: forced expiratory volume in one second; DLCOc: diffusing capacity for carbon monoxide corrected for hemoglobin; 6MWD: 6-minute walking distance; SUVmax: maximum standardized uptake value on ^{18}F-fluorodeoxyglucose by positron emission tomography; ACE: angiotensin-converting enzyme; sIL-2R: soluble interleukin-2 receptor.

Prior to initiation of infliximab, stable or deteriorating pulmonary function was seen in the total cohort (Figure 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 six 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 fiber neuropathy had subjective improvement of symptoms.

Table 2. Baseline disease parameters and change after 26 weeks of infliximab treatment in patients with pulmonary treatment indication.

	Baseline	Change after infliximab treatment
Subjects, n	28	
Pulmonary function parameters		
FVC, % predicted	73.6	+6.6
FEV1, % predicted	55.8	+5.8
DLCOc, % predicted	56.6	+4.1
6MWD, % 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
ACE, U/L	86.2 ± 46.3	-21.8 ± 43.3
ACE Z-score	3.7 ± 3.9	-1.78 ± 3.33
sIL-2R, pg/mL	7631 ± 4259	-3955 ± 3883

Data are presented as mean or mean ± SD unless otherwise stated. FVC: forced vital capacity; FEV1: forced expiratory volume in one second; DLCOc: diffusing capacity for carbon monoxide corrected for hemoglobin; 6MWD: 6-minute walking distance; SUVmax: maximum standardized uptake value on ^{18}F-fluorodeoxyglucose by positron emission tomography; ACE: angiotensin-converting enzyme; sIL-2R: soluble interleukin-2 receptor.

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 usable in 49 patients, 30 (61%) of whom had levels > 68 U/L. When measuring disease activity by means of ^{18}F-FDG PET scan before and after 26 weeks of treatment we found a 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 ($p<0.0001$) (Figure 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/L, respectively).

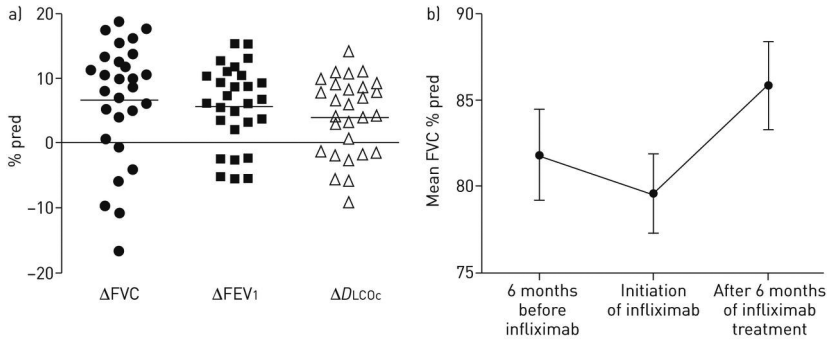


Figure 2. Pulmonary response in sarcoidosis.
a) Change in pulmonary function after 26 weeks of infliximab therapy in patients with a pulmonary treatment indication (change in % predicted), b) mean forced vital capacity (FVC) six months before initiation and during 26 weeks of infliximab therapy in the total cohort (% predicted). Bars represent SEM. FEV1: forced expiratory volume in one second; DLCOc: diffusing capacity of the lung for carbon monoxide corrected for hemoglobin; IFX: infliximab.

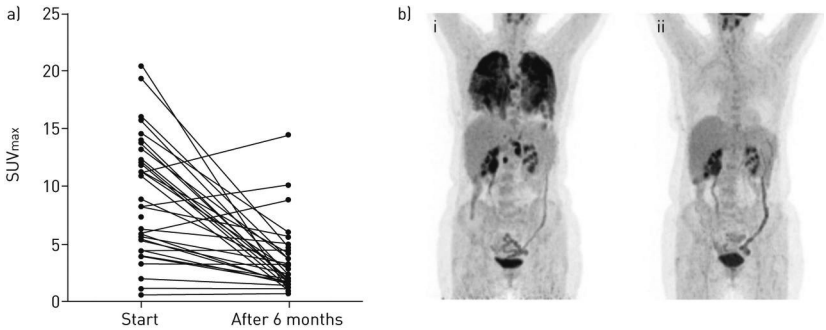


Figure 3. ^{18}F-fluorodeoxyglucose (FDG) by positron emission tomography (PET) activity during treatment.
a) Maximum standardized uptake value (SUVmax) on ^{18}F-FDG PET scan of the target organ at initiation and after 26 weeks of infliximab treatment, b) example of an ^{18}F-FDG PET scan in a patient with pulmonary sarcoidosis i) before and ii) after 26 weeks of infliximab treatment.

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 of 0.62 (p=0.0004) (Figure 4). Linear regression analysis, including the parameters SUVmax of the parenchyma and FVC at treatment initiation, predicted that FVC would 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 deterioration of lung function measured by FVC (Figure 2A) to the other patients, SUVmax of the pulmonary parenchyma and sIL-2R at baseline were found to be significantly different. No significant correlation with ACE, pulmonary function tests, 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). The dose of concomitant immunosuppressive drugs was increased in none of the patients.

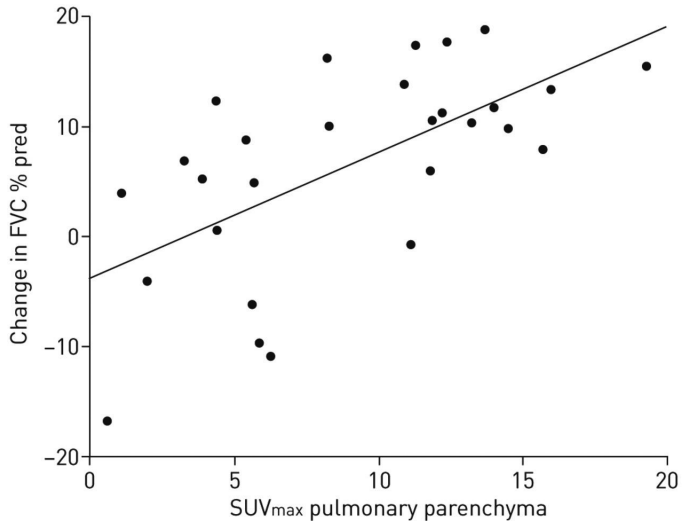


Figure 4. ^{18}F-fluorodeoxyglucose (FDG) by positron emission tomography (PET) activity and improvement in pulmonary function.
Correlation between high activity of pulmonary parenchyma on ^{18}F-FDG PET scan (maximum standardized uptake value (SUVmax)) at baseline and improvement in forced vital capacity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

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 a 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) after 26 weeks of treatment.

Composite overall response rate

When evaluating response after 26 weeks of treatment as a composite of three categories (organ function, inflammatory activity and quality of life) we found a very high response rate (Figure 5).

An excellent or good response was seen in 79% of patients, indicating clinically relevant improvement in at least two out of three categories. 17% of patients had a partial response, indicating improvement in one category. 4% of patients did not respond. When dividing response into the three categories, we found that 69% responded on the functional category, 79% on the inflammation category and 67% on the quality-of-life category. Patient characteristics, such as age, race, sex and disease duration did not predict overall response.

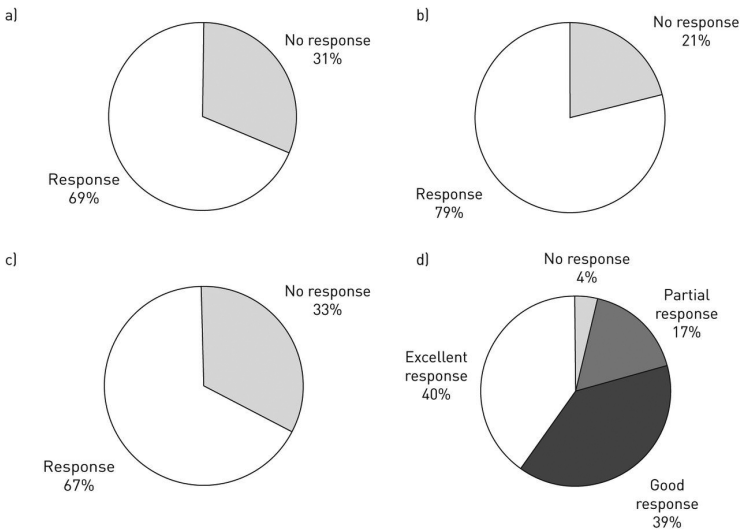


Figure 5. Response after 26 weeks of infliximab therapy.
Response was measured in a) organ function, b) inflammation, c) quality of life; d) composite overall response. Excellent responders showed marked improvement in all three categories; good responders in two categories; and partial responders in one category. Nonresponders showed no marked improvement on any of the three categories.

Infliximab trough levels and antibodies against infliximab

Infliximab trough levels showed high interindividual variation, but intraindividual variation was low throughout the 26 weeks of 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 mg/mL, partial responders a mean trough level of 17.4 mg/mL and nonresponders a mean trough level of 27.5 mg/mL.

Three patients showed continuously undetectable trough levels of infliximab. 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 antibodies against infliximab 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 7.5 mg/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 the intensive care unit 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 died 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 (n=2), dizziness (n=1), edema (n=3) and joint pain (n=2). 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 mean improvement of 6.6% predicted in FVC and a very high overall response rate.

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-inflammatory drug, probably finds more of its target in patients with higher inflammatory activity compared with 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 the disease activity markers ACE and sIL-2R. At initiation of infliximab therapy, mean levels of ACE and sIL-2R were 89.7 U/L and 8824 pg/mL, respectively (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. In our study, 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 improvement in FVC and high response rate found in our study compared to the only large RCT could be the difference in interval between infusions (4 vs 6 weeks). Infliximab levels were higher in our study than in the RCT (18.0 vs 7.5 mg/mL) and also higher than those reported in other immune-mediated inflammatory diseases [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, moreover, could serve to reduce the unfortunately considerable costs of treatment with biologicals such as infliximab. Low trough levels were observed in just three patients during the first 26 weeks of treatment. It remains possible that low trough levels due to the presence of antibodies against infliximab will occur more frequently after long-term treatment. Furthermore, concurrent use of methotrexate may have contributed to prevention of antibodies against infliximab formation in some patients, even though two out of the three patients with antibodies against infliximab also received methotrexate.

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 those 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 the evaluation of treatment for rare diseases [39].

Previous studies have focused mainly on response of functional parameters rather than parameters regarding inflammatory activity or quality of life [17,31]. Reduction of inflammation,

by infliximab, can be of clinical importance in possibly preventing future organ damage. Quality of life is an increasingly important, but under-reported outcome measure in sarcoidosis [40], which we therefore did include in the composite score. The combination of function, inflammation and quality of life (on a visual analogue scale) resembles the three categories also used in rheumatology in the disease activity score-28 [41,42]. A limitation of our composite overall response score is that deterioration in one category, when another category is improving, is not taken into account. Therefore, this composite overall response should be interpreted with caution and the response to the individual categories depicted in Figure 5A-C should be regarded as leading. 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 ^{18}F-FDG PET and biomarkers in most patients in the study. Hereby we were able to show better results compared to the large RCT [17]. However, the observed correlation between ^{18}F-FDG PET and pulmonary improvement could hypothetically have been even stronger when patients without disease activity would have been included.

The pharmaceutical industry has shown no interest in obtaining registration of approval by the US 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 the additional consequence of 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 the current fixed dosing regimen, levels of infliximab are high, suggesting room for dose reduction and associated cost reduction, *e.g.* by a flexible dosing regimen based on infliximab levels.

ACKNOWLEDGEMENTS

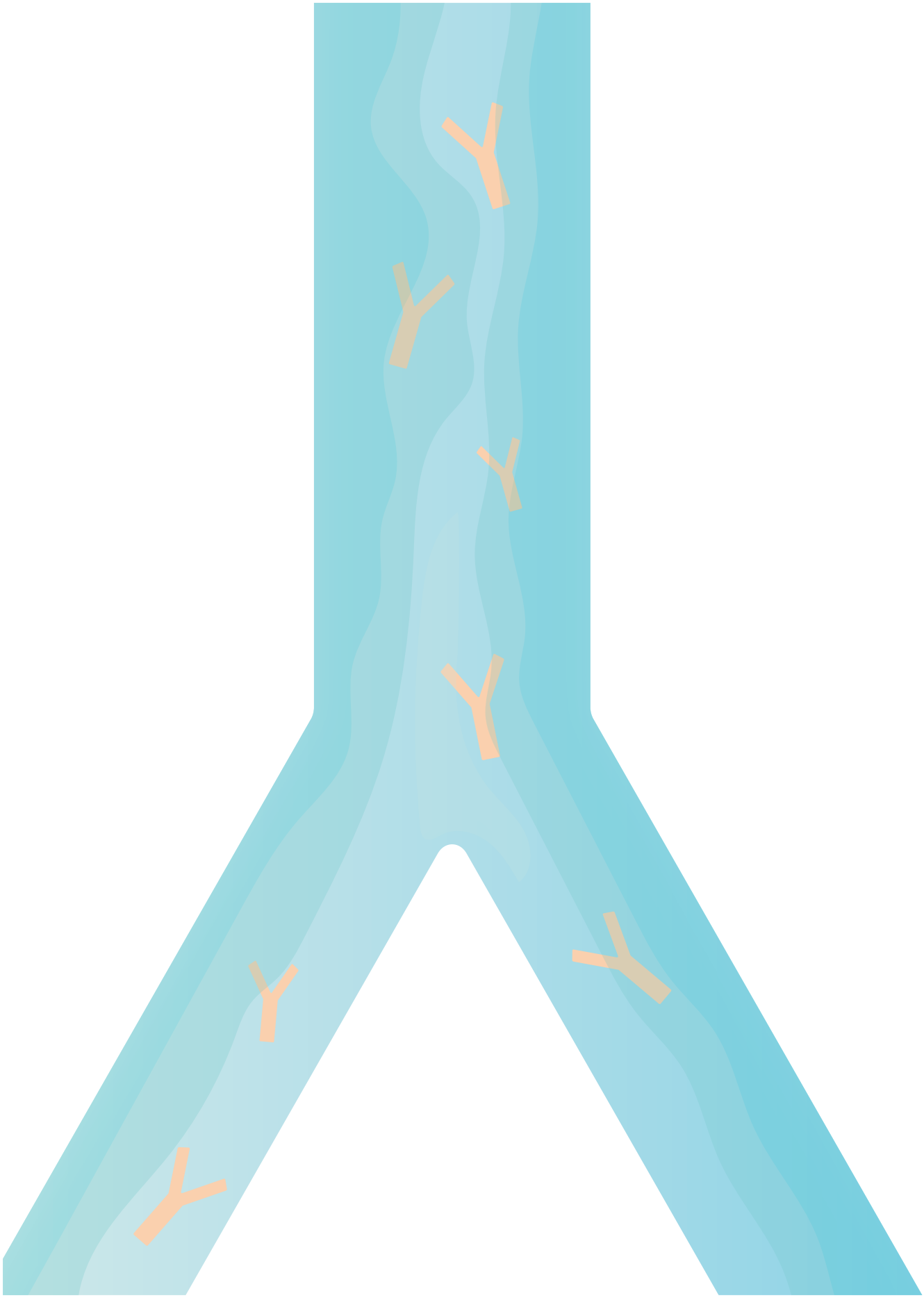
The authors would like to thank Pieter Zanen (University Medical Center Utrecht, Utrecht, The Netherlands) for his excellent statistical advice.

REFERENCES

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
2. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* 2011; 305: 391-399.
3. Judson MA, Baughman RP, Thompson BW, Teirstein AS, Terrin ML, Rossman MD, Yeager Jr H, McLennan G, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki BA, Weinberger SE, Knatterud GL, Cherniak R, ACCESS Research Group. Two year prognosis of sarcoidosis: the ACCESS experience. *Sarcoidosis Vasc Diffuse Lung Dis* 2003; 20: 204-211.
4. Grutters JC, van den Bosch JM. Corticosteroid treatment in sarcoidosis. *Eur Respir J* 2006; 28: 627-636.
5. Paramothayan NS, Lasserson TJ, Jones PW. Corticosteroids for pulmonary sarcoidosis. *Cochrane Database Syst Rev* 2005; (2): CD001114.
6. Vorselaars AD, van Moorsel CH, Deneer VH, Grutters JC. Current therapy in sarcoidosis, the role of existing drugs and future medicine. *Inflamm Allergy Drug Targets* 2013; 12: 369-377.
7. Baughman RP, Winget DB, Lower EE. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. *Sarcoidosis Vasc Diffuse Lung Dis* 2000; 17: 60-66.
8. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. *Arch Intern Med* 1995; 155: 846-851.
9. Cremers JP, Drent M, Bast A, Shigemitsu H, Baughman RP, Valeyre D, Sweiss NJ, Jansen TL. Multinational evidence-based World Association of Sarcoidosis and Other Granulomatous Disorders recommendations for the use of methotrexate in sarcoidosis: integrating systematic literature research and expert opinion of sarcoidologists worldwide. *Curr Opin Pulm Med* 2013; 19: 545-561.
10. Vorselaars AD, Wuyts WA, Vorselaars VM, Zanen P, Deneer VH, Veltkamp M, Thomeer M, van Moorsel CH, Grutters JC. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* 2013; 144: 805-812.
11. Drent M, Cremers J, Jansen T, Baughman R. Practical eminence and experience-based recommendations for use of TNF-α inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
12. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998; 41: 1552-1563.
13. Chaudhari U, Romano P, Mulcahy LD, Dooley LT, Baker DG, Gottlieb AB. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet* 2001; 357: 1842-1847.
14. D'Haens G, Van Deventer S, Van Hogezand R, Chalmers D, Kothe C, Baert F, Braakman T, Schaible T, Geboes K, Rutgeerts P. Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: A European multicenter trial. *Gastroenterology* 1999; 116: 1029-1034.
15. van Rijswijk HN, Vorselaars AD, Ruven HJ, Keijsers RG, Zanen P, Korenromp IH, Grutters JC. Changes in disease activity, lung function and quality of life in patients with refractory sarcoidosis after anti-TNF treatment. *Expert opinion on orphan drugs* 2013; 1: 437-443.
16. Hostettler KE, Studler U, Tamm M, Brutsche MH. Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218-224.
17. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
18. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, Drent M, Centocor T48 Sarcoidosis Investigators. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J* 2008; 31: 1189-1196.

19. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. *Lancet* 2014; 383: 1155-1167.
20. Maneiro JR, Salgado E, Gomez-Reino JJ, Carmona L, BIOBADASER Study Group. Efficacy and safety of TNF antagonists in sarcoidosis: data from the Spanish registry of biologics BIOBADASER and a systematic review. *Semin Arthritis Rheum* 2012; 42: 89-103.
21. Carneiro AV, Costa J. Off-label prescription: practice and problems. *Rev Port Cardiol* 2013; 32: 681-686.
22. Zimmermann A, Dubaniewicz A, Slominski JM. Pharmacotherapy for sarcoidosis: an example of an off-label procedure. *Adv Exp Med Biol* 2013; 755: 251-256.
23. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijsers RG, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J* 2014; 43: 602-609.
24. Garces S, Demengeot J, Benito-Garcia E. The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis. *Ann Rheum Dis* 2013; 72: 1947-1955.
25. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 34: 51-58.
26. Wang W, Wang EQ, Balthasar JP. Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther* 2008; 84: 548-558.
27. Hoitsma E, De Vries J, Drent M. The small fiber neuropathy screening list: Construction and cross-validation in sarcoidosis. *Respir Med* 2011; 105: 95-100.
28. Baughman RP, Nunes H, Sweiss NJ, Lower EE. Established and experimental medical therapy of pulmonary sarcoidosis. *Eur Respir J* 2013; 41: 1424-1438.
29. Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BA, Aarden L. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 704-707.
30. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 711-715.
31. Wijnen PA, Cremers JP, Nelemans PJ, Erckens RJ, Hoitsma E, Jansen TL, Bekers O, Drent M. Association of the TNF-alpha G-308A polymorphism with TNF-inhibitor response in sarcoidosis. *Eur Respir J* 2014; 43: 1730-1739.
32. Keir G, Wells AU. Assessing pulmonary disease and response to therapy: which test? *Semin Respir Crit Care Med* 2010; 31: 409-418.
33. Adams H, Keijsers RG, Korenromp IH, Grutters JC. FDG PET for gauging of sarcoid disease activity. *Semin Respir Crit Care Med* 2014; 35: 352-361.
34. Keijsers RG, Verzijlbergen FJ, Oyen WJ, van den Bosch JM, Ruven HJ, van Velzen-Blad H, Grutters JC. 18F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis. *Eur J Nucl Med Mol Imaging* 2009; 36: 1131-1137.
35. Bortlik M, Duricova D, Malickova K, Machkova N, Bouzkova E, Hrdlicka L, Komarek A, Lukas M. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013; 7: 736-743.
36. van der Maas A, van den Bemt BJ, Wolbink G, van den Hoogen FH, van Riel PL, den Broeder AA. Low infliximab serum trough levels and anti-infliximab antibodies are prevalent in rheumatoid arthritis patients treated with infliximab in daily clinical practice: results of an observational cohort study. *BMC Musculoskelet Disord* 2012; 13: 184.
37. Takahashi H, Tsuji H, Ishida-Yamamoto A, Iizuka H. Plasma trough levels of adalimumab and infliximab in terms of clinical efficacy during the treatment of psoriasis. *J Dermatol* 2013; 40: 39-42.
38. Vorselaars AD, Cremers JP, Grutters JC, Drent M. Cytotoxic agents in sarcoidosis: which one should we choose? *Curr Opin Pulm Med* 2014; 20: 479-487.
39. Korn EL, McShane LM, Freidlin B. Statistical challenges in the evaluation of treatments for small patient populations. *Sci Transl Med* 2013; 5: 178sr3.
40. Korenromp IH, van de Laar MA. Health-related quality of life in sarcoidosis. *Curr Opin Pulm Med* 2014; 20: 503-507.

41. Eng G, Stoltenberg MB, Szkudlarek M, Bouchelouche PN, Christensen R, Bliddal H, Marie Bartels E. Efficacy of treatment intensification with adalimumab, etanercept and infliximab in rheumatoid arthritis: a systematic review of cohort studies with focus on dose. *Semin Arthritis Rheum* 2013; 43: 144-151.
42. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-48.
43. Judson MA, Baughman RP, Costabel U, Drent M, Gibson KF, Raghu G, Shigemitsu H, Barney JB, Culver DA, Hamzeh NY, Wijsenbeek MS, Albera C, Huizar I, Agarwal P, Brodmerkel C, Watt R, Barnathan ES. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. *Eur Respir J* 2014; 44:1296-307.



4

Long-term infliximab treatment in sarcoidosis: effect after six months is maintained during two years of treatment

Heleen A Crommelin
Adriane DM Vorselaars
Coline HM van Moorsel
Vera HM Deneer
Jan C Grutters

Submitted

ABSTRACT

Background: When sarcoidosis patients are unresponsive to conventional first- and second-line treatment, infliximab is considered an important next treatment option. Although long-term treatment is used in clinical practice, no prospective data on long-term treatment response are available.

Methods: This prospective observational cohort study included severe sarcoidosis patients treated with 5 mg/kg infliximab intravenously at week 0, 2 and every four weeks thereafter. Organ function and inflammatory biomarkers were evaluated every six and three months, respectively, for up to two years. In case of clinical stability and low levels of inflammatory parameters, the dosing interval was lengthened based upon the physician's judgement. The study was registered in the Dutch Trial Register under number NTR3895 (www.trialregister.nl).

Results: Infliximab was initiated in 56 patients and was continued for at least one year in 37 patients (66%) and for at least two years in 26 patients (46%). Pulmonary function tests showed greatest improvement after six months and inflammatory biomarkers after three months of treatment. In patients where the dosing interval was lengthened (n=14), organ function and inflammatory biomarkers remained stable.

Conclusions: The positive effect of six months treatment with infliximab in severe refractory sarcoidosis can be maintained for at least two years under prolonged treatment. Furthermore, data show that the dosing interval between infusions can be safely lengthened in selected patients with clinical stability and low inflammatory markers.

INTRODUCTION

Sarcoidosis is a multi-organ disease, characterized by non-caseating granuloma formation. The etiology of the disease remains unclear, but studies support a role for both genetic involvement and environmental factors [1]. The disease can be self-limiting, but in a significant subgroup of patients the disease follows a chronic course [2,3]. Corticosteroid therapy is usually considered to be first-line therapy in sarcoidosis, but prolonged use can cause serious side effects [4]. Second-line therapy usually involves cytotoxic and immunomodulatory agents such as methotrexate and azathioprine [5-9]. However, some patients are refractory to these agents or may suffer from serious side effects. In these patients biological tumor necrosis factor (TNF) inhibitors have been introduced as third-line therapy [10-13]. Infliximab, a biological drug, specifically targets TNF and inhibits it from acting as a key messenger molecule in the inflammatory process. We have previously shown that infliximab is effective in severe sarcoidosis after six months of treatment [12]. Unfortunately, infliximab does not cure sarcoidosis: in a study by Vorselaars *et al.* [14] it was shown that 62% of patients relapsed after discontinuation of therapy, with a median time to relapse of 11 months. Therefore, patients are mostly treated with infliximab for a long period of time.

To date, data on long-term efficacy of infliximab in sarcoidosis is scarce: only two small retrospective studies have provided data on clinical follow-up of patients over one year of infliximab therapy [15,16]. Data on maintenance of the effect during infliximab treatment and reaction to change of dosing regimens in sarcoidosis have never been published.

According to empirically based practice, infliximab treatment may be slowly tapered by lengthening the dosing interval between infusions from four to six or eight weeks in patients with stable clinical and inflammatory parameters [17]. However, no data on the effect of this treatment modification is currently available.

This is the first prospective study to evaluate results of two years of follow-up of a well-described cohort of patients with severe sarcoidosis treated with infliximab.

METHODS

Study subjects

All sarcoidosis patients who started infliximab treatment at St Antonius Hospital (Nieuwegein, The Netherlands) as part of routine patient care between January 2011 and April 2013 were invited to participate in this study. The basic study design has been reported previously [12]. Patients were found eligible for infliximab treatment in case of severe sarcoidosis refractory to first- and second-line treatment or suffering from severe side effects from first- and second-line agents. Diagnosis of sarcoidosis was made according to American Thoracic Society/European Respiratory Society criteria [1]. Exclusion criteria were vaccination with live or bacterial vaccines 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 hospital's medical research ethics committee (VCMO) approved the study (registration/trial number LTME/R-10.13A, acronym INFLIXIMAB) and patients gave written informed consent. The study was registered in the Dutch Trial Register under number NTR3895 (Dutch Trial Register; www.trialregister.nl).

Treatment

Patients received infliximab intravenously following a standard protocol starting with 5 mg/kg bodyweight at weeks 0, 2 and then every four weeks. According to the local treatment protocol, in case of clinical stability and low inflammatory biomarkers compared to baseline, the dosing interval was prolonged to six and later eight weeks based upon the physician's judgement after at least six months of treatment. Patient follow-up continued up to two years after treatment initiation. Functional evaluation of the index organ was assessed every six months. Forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (DLCOc) were evaluated in pulmonary sarcoidosis. The small fiber neuropathy short list (SFN-SL) questionnaire and assessment by an experienced neurologist were used in small fiber neuropathy (SFN). Assessment by the neurologist was used in sarcoidosis of the central nervous system. Left ventricular ejection fraction on transthoracic echocardiogram, myocardial uptake on ^{18}F-fluoro-deoxyglucose by positron emission tomography (^{18}F-FDG PET) scan and the assessment by an experienced cardiologist were used in cardiac sarcoidosis. Cutaneous sarcoidosis was evaluated by visual examination of the lesions. Osseous sarcoidosis and myositis were evaluated by activity on ^{18}F-FDG PET scan.

Inflammatory biomarkers soluble interleukin-2 receptor (sIL-2R) and angiotensin-converting enzyme (ACE) were measured every three months. The initial treatment results after the first six months of treatment have been previously described [12].

Pulmonary function testing and inflammatory serum markers

Pulmonary function tests were performed using the Master Screen Body system (Jaeger ms-pft analysis unit, Würtzburg, Germany). Quantification of sIL-2R levels (levels > 3000 pg/mL considered elevated) was performed in serum with enzyme immunoassays according to the manufacturer's instructions (Diaclone; Sanquin, Amsterdam, The Netherlands). 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). sIL-2R levels from patients with kidney dysfunction were excluded from analysis [18], and ACE values of patients using ACE-inhibitors were also excluded.

Antibodies against infliximab and infliximab trough serum levels

Antibodies against infliximab were determined as part of routine care when the treating physician suspected presence of antibodies against infliximab. These antibodies were measured using radioimmunoassay by Sanquin, The Netherlands [19].

Serum samples for determining infliximab concentrations were drawn just before infusion of every new dose during the first six months and thereafter every three months. Infliximab trough serum levels were measured using an ELISA (Sanquin, The Netherlands) [20]. This ELISA only detects infliximab that is able to bind to TNF. It does not detect immune complexes consisting of infliximab and TNF or infliximab bound to neutralizing antibodies against infliximab.

Statistics

Changes before and after treatment were compared using two-tailed paired t-tests. Groups were compared using two-tailed independent sample t-tests. Statistical analyses were performed using SPSS for Windows (version 24.0 IBM, Armonk, NY, USA). Graphs were created using Graphpad Prism 6.05 (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered significant.

RESULTS

Patients

A total of the 56 patients started on infliximab therapy of which 37 patients (66%) continued infliximab for at least one year and 26 patients (46%) for at least two years (Figure 1). A total of 30/56 (54%) patients discontinued infliximab for the following reasons: 14/56 (25%) due to adverse events (including eight infections and four infusion reactions), 8/56 (14%) due to disease progression, 7/56 (13%) due to disease remission and 1/56 (2%) for undisclosed reasons. Treatment discontinuation occurred most often during the first six months of treatment (Figure 2). Baseline characteristics of patients treated at least one year (n=37) are shown in Table 1.

Long-term evaluation of functional response

At baseline, mean FVC in patients with a pulmonary treatment indication (n=21) was 72.9% predicted. Mean change in FVC from baseline was +8.1% predicted after six months of treatment and remained stable whilst continuing treatment for two years. The change in FVC from baseline was highly variable between patients and ranged between -20 (after one year) and +25% predicted (after 18 months, Figure 3A). During follow-up the number of patients with > 5% predicted improvement in FVC decreased slightly. After six months of treatment, 17/21 patients (81%) showed improvement in FVC of ≥ 5% predicted. After one year of treatment improvement in FVC of 5% predicted or more was present in 15/21 patients (71%) and in 11/14 patients (79%) after two years of treatment.

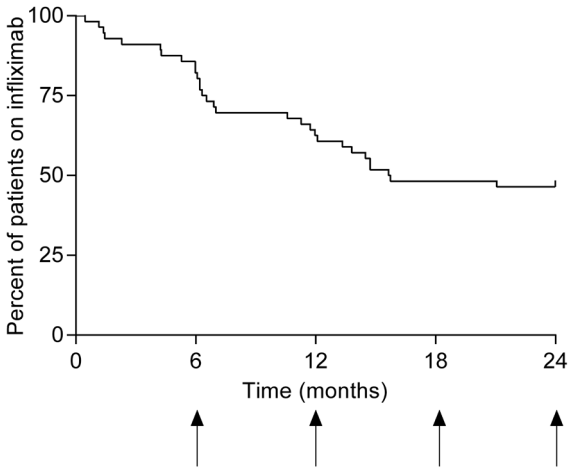


Figure 1. Percent of patients continuing infliximab treatment**.**
Arrows indicate evaluation of treatment response according to treatment protocol.

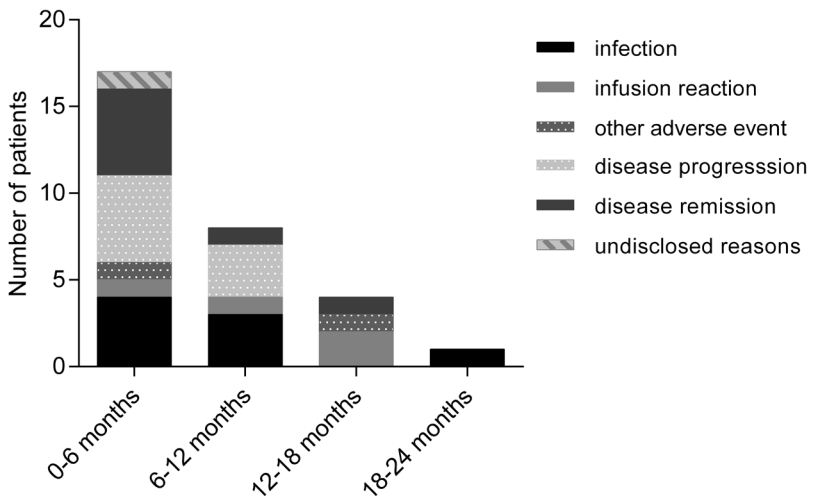


Figure 2. Reasons for discontinuation of infliximab treatment.
Patients were initially treated for six months. Every six months a decision regarding treatment continuation/ discontinuation was made based upon treatment response and adverse events. Treatment discontinuation because of remission was evaluated every six months.

Table 1. Baseline characteristics of patients treated with infliximab for at least one year.

Subjects, n	37
Demographics	
Age, yrs	47 (range 29-68)
Male sex	23 (62.2)
Caucasian	33 (89.2)
Diagnosis biopsy proven	35 (94.6)
Disease duration, yrs	6.4 ± 6.3
Medication use prior to initiation of infliximab	
Corticosteroids	35 (94.6)
Methotrexate	33 (89.2)
Azathioprine	4 (10.8)
Leflunomide	1 (2.7)
Plaquenil	5 (13.5)
Anti-TNF treatment	10 (27.0)
None	0 (0.0)
Use of ≥ 2 different drugs prior to infliximab	33 (89.2)
Treatment indication, n (%)	
Pulmonary	21 (56.8)
Extrapulmonary	16 (43.2)
Small fiber neuropathy	7 (18.9)
Cutaneous	3 (8.1)
Cardiac	2 (5.4)
Central nervous system	1 (2.7)
Hypercalcemia	1 (2.7)
Myositis	1 (2.7)
Ossal	1 (2.7)
Baseline values	
FVC, % predicted	79.6 ± 17.3
DLCOc, % predicted	59.8 ± 15.0
sIL-2R, pg/mL	8721 ± 5559
ACE, U/L	93 ± 48
SUVmax	9.7 ± 5.0

Data are presented as n (%) or mean ± SD unless otherwise stated. TNF: tumor necrosis factor; FVC: forced vital capacity; DLCOc: diffusing capacity for carbon monoxide corrected for hemoglobin; sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme; SUVmax total: maximum standardized uptake value on ^{18}F-fluorodeoxyglucose by positron emission tomography scan.

Mean DLCOc at baseline in patients with a pulmonary treatment indication was 52.0% predicted. Compared to baseline the median change in DLCOc remained similar between +5.8 (after six months) and +8.0% predicted (after two years) throughout the treatment period. Change in DLCOc was highly variable between patients, ranging from -15 (after two years) to +22% predicted (also after two years, Figure 3B). After six months of treatment, DLCOc improvement of ≥ 5% predicted occurred in 13/21 patients (62%). After one year of treatment this was again 13/21 patients (62%) and after two years of treatment in 10/14 patients (71%).

In all three patients with cutaneous sarcoidosis skin lesions the response was maintained during follow-up. Four out of seven patients with SFN showed a clinical response after twelve months of treatment and all four patients maintained clinical response according to the judgement of the physician.

Inflammatory biomarker change during long-term treatment

In the first three months of treatment the mean level of inflammatory biomarker sIL-2R decreased significantly to near normal levels (< 3000 pg/mL) from 8721 pg/mL (n=34) to 4584 pg/mL (p<0.001, n=31). After three months sIL-2R levels remained stable for up to two years (Figure 3C). sIL-2R levels were elevated (> 3000 pg/mL) at baseline in 31/34 patients (91%), which persisted in 17/23 patients (74%) after two years of treatment.

Mean ACE at baseline was 93 U/L (n=31) and decreased significantly to 56 U/L (p<0.001, n=27) after three months and continued to decrease ultimately to 50 U/L (n=23) after two years of treatment (Figure 3D). At baseline ACE corrected for genotype was elevated in 19/31 patients (61%) which decreased to 7/23 patients (30%) after two years of treatment.

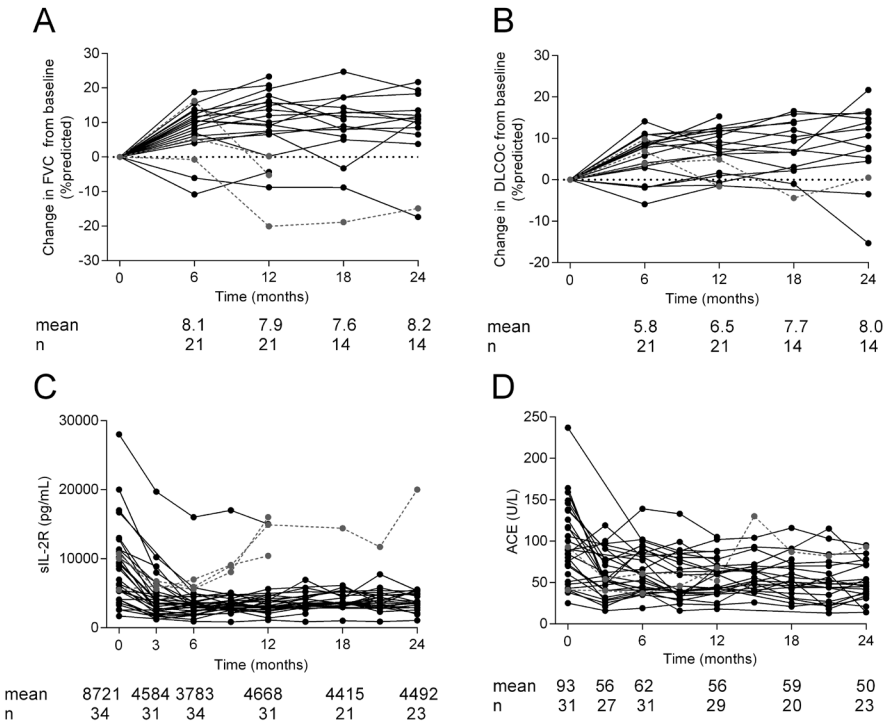


Figure 3. Course of pulmonary function tests forced vital capacity (FVC, panel A) and diffusing capacity of carbon monoxide corrected for hemoglobin (DLCOc, panel B) in patients with a pulmonary treatment indication. Course of inflammatory biomarkers soluble interleukin-2 receptor (sIL-2R, panel C) and angiotensin-converting enzyme (ACE, panel D) in all patients over two years of follow-up after initiation of infliximab therapy. Grey dashed lines represent patients positive for antibodies against infliximab.

Development of antibodies against infliximab

Seventeen patients were tested for the presence of antibodies against infliximab. Three of these patients (18%) were tested positive. All three patients were prescribed concomitant immunosuppressive drugs: two patients received methotrexate 7.5 mg/week and one patient prednisone 20 mg/day. Eventually, all three patients discontinued infliximab treatment: two patients discontinued treatment because of infusion reactions and loss of response and one patient because of a suspected aspergillum infection. FVC and DLCOc deteriorated in two patients and were stable in one patient. The three patients showed increasing sIL-2R levels and increasing ACE levels in two patients, the third patient used an ACE-inhibitor (Figure 3 grey dashed lines).

Lengthening the dosing interval

According to the local treatment protocol lengthening the dosing interval between infliximab infusions from four to six weeks or more is considered an option in patients with clinical stability and low levels of inflammatory parameters compared to baseline after at least six months of treatment. In 14/38 patients (37%) the dosing interval was prolonged to six weeks. For none of the patients for whom the dosing interval was lengthened was this decision reversed. The mean infliximab treatment duration at the time the dosing interval was lengthened was 12.5 months (range 6.2-18.4 months) and the mean follow-up after lengthening the dosing interval was 9.9 months (range 4.4-17.8 months) (Table 2). A total of 6/14 (43%) patients started infliximab because of extrapulmonary disease. All patients had reduced FVC and/or DLCOc of less than 80% predicted at baseline. All patients but one had responded to treatment after the first six months. The patient who did not respond was treated because of myositis.

Patients for whom the dosing interval was lengthened, showed significantly lower total maximum standardized uptake value (SUVmax) on ^{18}F-FDG PET scan after the first six months of infliximab treatment compared to patients for whom the dosing interval remained four weeks (2.3 vs 4.0, p=0.033). Total SUVmax and sIL-2R at baseline and sIL-2R after six months of infliximab treatment did not differ significantly between the two groups.

FVC and levels for inflammatory biomarkers just prior to lengthening the dosing interval were similar compared to values approximately six months later. DLCOc did show a statistically significant increase: mean DLCOc increased from 62 to 65% predicted (p=0.02, Figure 4 and Table 2). Organ function in patients with an extrapulmonary treatment indication remained stable.

In six of the 14 patients for whom dosing interval between infusions was lengthened to six weeks, the dosing interval was further lengthened thereafter to eight weeks, but follow-up time was insufficient to evaluate functional and inflammatory parameters.

Table 2. Characteristics of patients for whom the dosing interval between infusions was lengthened.

Characteristic	Baseline values at the time of start lengthening the dosing interval	Six months after lengthening the dosing interval
Treatment duration at the time dosing interval was lengthened, months	12.5 ± 4.0	
Follow-up after lengthening the dosing interval, months	9.9 ± 4.2	
Baseline values		
FVC, % predicted	90.8 ± 15.3*	91.4 ± 15.4*
DLCOc, % predicted	62.3 ± 15.2**	64.7 ± 15.2**
sIL-2R, pg/mL	3758 ± 1623**	3659 ± 1567**
ACE, U/L	45 ± 16***	42 ± 19***

Values stated as mean ± SD. FVC: forced vital capacity; DLCOc: diffusing capacity for carbon monoxide corrected for hemoglobin; sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme; * n=13; ** n=12; *** n=8.

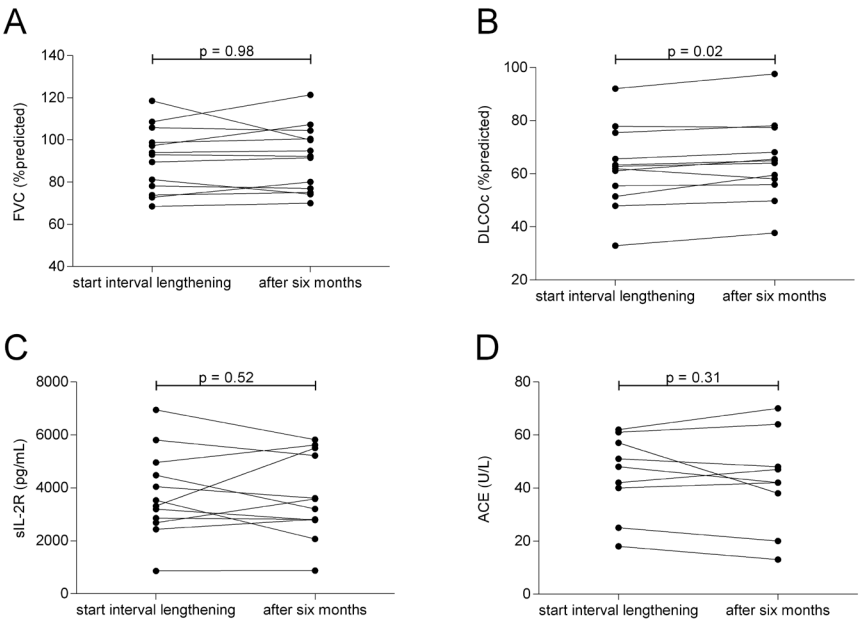


Figure 4. Pulmonary function tests forced vital capacity (FVC, panel A) and diffusing capacity of carbon monoxide corrected for hemoglobin (DLCOc, panel B) and inflammatory biomarkers soluble interleukin-2 receptor (sIL-2R, panel C) and angiotensin-converting enzyme (ACE, panel D) prior to and six months in all patients after lengthening the dosing interval.
No significant differences in FVC, sIL-2R and ACE were found between values at the start of interval lengthening compared to values six months later. DLCOc did change significantly, but this change was thought to be not clinically relevant. Paired t-test was used for statistical comparison.

In 13 patients for whom the dosing interval between infusions was lengthened the infliximab trough serum concentration was measured. In total, 34 samples were available with a range of 1 to 6 samples per patient. The mean infliximab trough concentration per patient was 9 µg/mL (Figure 5). The lowest concentration measured was 2 µg/mL. This level was measured in two patients. For both these patients pulmonary function and inflammatory biomarkers had not deteriorated six months after lengthening the dosing interval from four to six weeks.

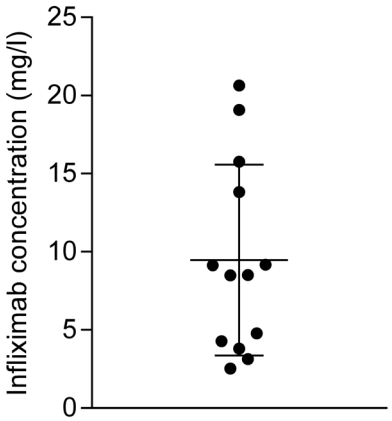


Figure 5. Infliximab trough serum concentration after lengthening the dosing interval between infliximab infusions from four to six weeks with mean and standard deviation.
Every dot represents the mean infliximab trough serum concentration in one patient.

DISCUSSION

Patients with severe sarcoidosis who are treated with infliximab often receive long-term therapy because of high relapse rates after discontinuation of therapy. Yet there is scant information on long-term efficacy and effect of dosing modifications of infliximab in sarcoidosis. This prospective, observational study in a well-described infliximab treated cohort with two years of detailed follow-up shows that the largest clinical improvement, measured by pulmonary function, occurs during the first six months of treatment. And the largest improvement in inflammatory biomarkers, which were measured more frequently, occurs during the first three months of infliximab treatment. This response is maintained during treatment prolongation for two years. Moreover, clinical and inflammatory parameters remain stable in patients for whom the dosing interval is lengthened in case of clinical stability and low inflammatory parameters compared to baseline.

Currently, there is no evidence-based protocol for the assessment of response to infliximab therapy in sarcoidosis. Previously, we have shown that decrease in sIL-2R after six months correlates with response after six months of treatment [21]. Here, we show that sIL-2R already

significantly decreases after three months of treatment. Therefore, it appears that evaluation of response to infliximab in sarcoidosis can be based on the change of this biomarker at three months of treatment, although this warrants further research.

When the dosing interval between infusions was lengthened from four to six weeks in selected patients with clinical stability and low inflammatory markers compared to baseline, pulmonary function and inflammatory biomarkers were found to remain stable. These findings support local sarcoidosis treatment protocols that in patients with clinical stability and low inflammatory parameters lengthening the dosing interval is a safe clinical practice [17]. Lengthening time between infusions reduces the number of visits patients have to make to their clinic and saves drug costs.

In the patients who remained clinically stable, including low sIL-2R levels, upon lengthening the dosing interval we observed infliximab trough concentrations as low as 2 µg/mL. This observation could either indicate that these subjects have finally entered a spontaneous remission state of their disease or only need small amounts of infliximab to maintain disease control. Several studies on inflammatory bowel disease suggest an added value of therapeutic drug monitoring of infliximab in current practice with evidence indicating a therapeutic window ranging from 3 to 7 µg/mL [22,23]. In future research in sarcoidosis patients tapering infliximab treatment, measuring infliximab serum concentration could provide an interesting new parameter.

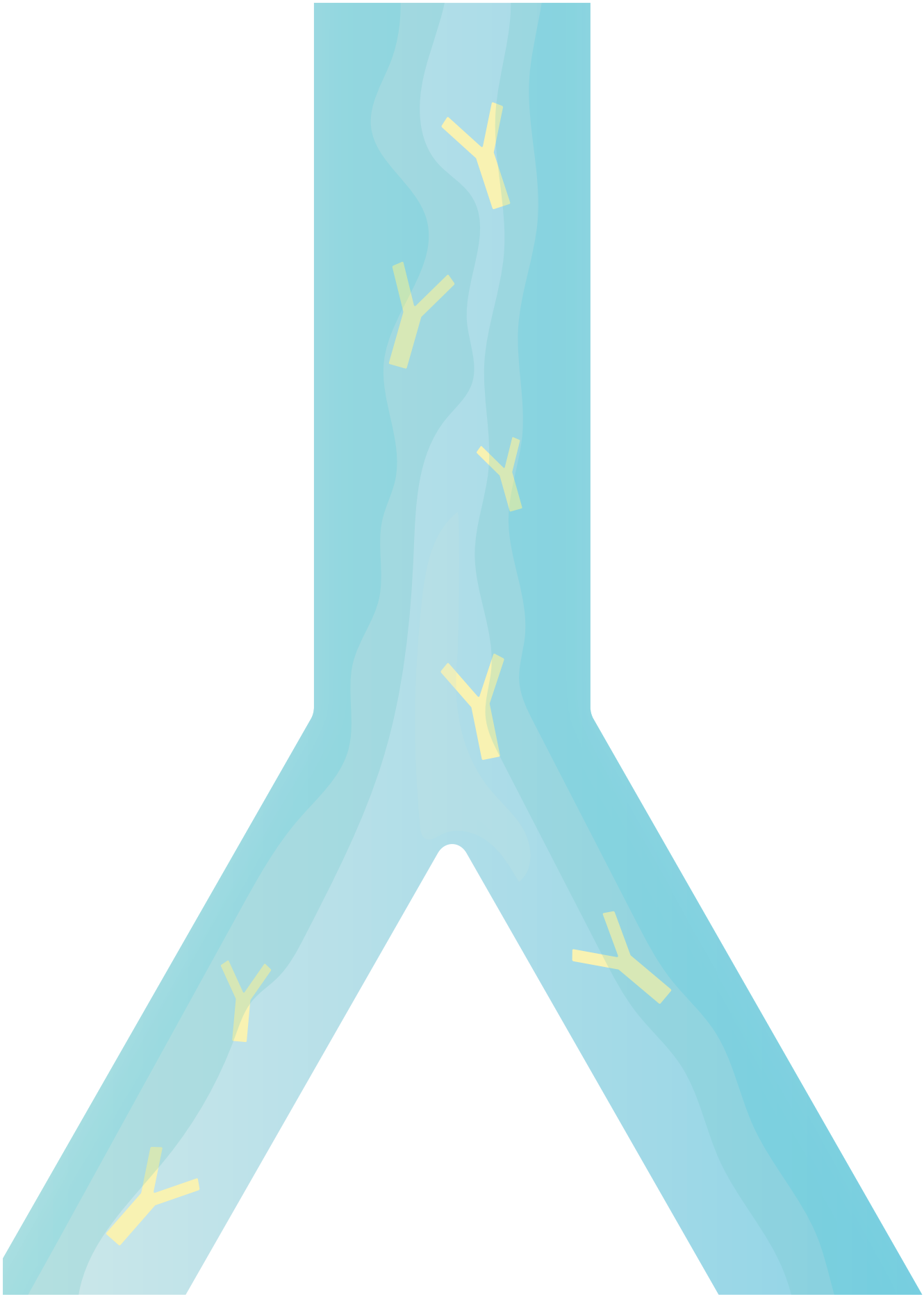
CONCLUSIONS

The positive effect of six months treatment with infliximab in severe refractory sarcoidosis can be maintained for at least two years under prolonged treatment. Furthermore, data show that the dosing interval between infusions can be safely lengthened in selected patients with clinical stability and low inflammatory markers.

REFERENCES

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
2. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* 2011; 305: 391-399.
3. Judson MA, Thompson BW, Rabin DL, Steimel J, Knattereud GL, Lackland DT, Rose C, Rand CS, Baughman RP, Teirstein AS, ACCESS Research Group. The diagnostic pathway to sarcoidosis. *Chest* 2003; 123: 406-412.
4. Vorselaars AD, van Moorsel CH, Deneer VH, Grutters JC. Current therapy in sarcoidosis, the role of existing drugs and future medicine. *Inflamm Allergy Drug Targets* 2013; 12: 369-377.
5. Baughman RP, Winget DB, Lower EE. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. *Sarcoidosis Vasc Diffuse Lung Dis* 2000; 17: 60-66.
6. Lower EE, Baughman RP. The use of low dose methotrexate in refractory sarcoidosis. *Am J Med Sci* 1990; 299: 153-157.
7. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. *Arch Intern Med* 1995; 155: 846-851.
8. Muller-Quernheim J, Kienast K, Held M, Pfeifer S, Costabel U. Treatment of chronic sarcoidosis with an azathioprine/prednisolone regimen. *Eur Respir J* 1999; 14: 1117-1122.
9. Lewis SJ, Ainslie GM, Bateman ED. Efficacy of azathioprine as second-line treatment in pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999; 16: 87-92.
10. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller Jr W, Sands BE. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006; 23: 201-208.
11. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
12. Vorselaars AD, Crommelin HA, Deneer VH, Meek B, Claessen AM, Keijsers RG, van Moorsel CH, Grutters JC. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J* 2015; 46: 175-185.
13. Crommelin HA, Vorselaars AD, van Moorsel CH, Korenromp IH, Deneer VH, Grutters JC. Anti-TNF therapeutics for the treatment of sarcoidosis. *Immunotherapy* 2014; 6: 1127-1143.
14. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijsers RG, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J* 2014; 43: 602-609.
15. Russell E, Luk F, Manocha S, Ho T, O'Connor C, Hussain H. Long term follow-up of infliximab efficacy in pulmonary and extra-pulmonary sarcoidosis refractory to conventional therapy. *Semin Arthritis Rheum* 2013; 43: 119-124.
16. Hostettler KE, Studler U, Tamm M, Brutsche MH. Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218-224.
17. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
18. Verwoerd A, Vorselaars AD, van Moorsel CH, Bos WJ, van Velzen-Blad H, Grutters JC. Discrepant elevation of sIL-2R levels in sarcoidosis patients with renal insufficiency. *Eur Respir J* 2015; 46: 277-280.
19. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 711-715.
20. Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BA, Aarden L. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 704-707.

21. Vorselaars AD, van Moorsel CH, Zanen P, Ruven HJ, Claessen AM, van Velzen-Blad H, Grutters JC. ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. *Respir Med* 2015; 109: 279-285.
22. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: Adding value to current practice. *J Clin Pharmacol* 2015; 55 Suppl 3: S39-50.
23. Vande Casteele N, Ferrante M, Van Assche G, Ballet V, Compernolle G, Van Steen K, Simoens S, Rutgeerts P, Gils A, Vermeire S. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015; 148: 1320-1329.



5

Efficacy of adalimumab in sarcoidosis patients who developed intolerance to infliximab

Heleen A Crommelin
Leone M van der Burg
Adriane DM Vorselaars
Marjolein Drent
Coline HM van Moorsel
Ger T Rijkers
Vera HM Deneer
Jan C Grutters

Respir Med. 2016 Jun;115:72-77.

ABSTRACT

Background: Tumor necrosis factor-alpha (TNF-α) inhibitors are regarded as the third-line therapy in sarcoidosis, the first choice generally being infliximab. To date, data regarding response to adalimumab in sarcoidosis patients intolerant to infliximab are lacking.

The objective of this retrospective observational study was to establish if adalimumab could achieve stabilization or improvement of the disease in refractory sarcoidosis patients who developed intolerance to infliximab.

Methods: Sarcoidosis patients referred to St Antonius Interstitial Lung Diseases Center of Excellence, Nieuwegein, The Netherlands, between January 2008 and April 2015 who switched from infliximab to adalimumab were included. Changes in organ function, inflammatory biomarker levels, and adverse events were retrieved from medical records.

Results: Out of 142 infliximab treated patients, 18 (13%) had to discontinue treatment due to antibody formation or severe adverse events and switched to adalimumab therapy. Organ function improved in seven patients (39%), was stable in six patients (33%), and worsened in five patients (28%) after twelve months of treatment or after six months if evaluation after twelve months was not available (n=4). In none of the patients biomarker levels of soluble interleukin-2 receptor (sIL-2R) deteriorated. Median decrease in sIL-2R was 3614 pg/mL. The most frequently reported adverse event was infection (n=10).

Conclusions: Adalimumab is an effective alternative for patients intolerant to infliximab. The switch to adalimumab achieved clinical improvement in 39% and stabilization in 33% of the patients intolerant to infliximab. Further research is needed to develop guidelines on how to use adalimumab for sarcoidosis in terms of dosing regimen.

INTRODUCTION

Sarcoidosis is a systemic disease characterized by the formation of non-necrotizing granulomas. In 30 - 60% of patients, the disease is self-limiting and no systemic treatment is required [1]. In case of treatment failure or serious adverse events with the first-line treatment with glucocorticosteroids, second-line agents are methotrexate (MTX) and azathioprine [2-9]. Finally, third-line therapy consists of monoclonal antibodies targeted specifically at tumor necrosis factor-alpha (TNF-α), a key cytokine in sarcoidosis [10-12]. Of these anti-TNF-α agents, the most extensively studied and most often used in therapy is infliximab [13-17]. However, patients may develop antibodies against infliximab, especially because of its chimeric nature. In diseases other than sarcoidosis it has been shown that patients who tested positive for antibodies against infliximab have an increased risk of allergic reaction and a 3-fold higher risk of loss of clinical response [18-21]. In sarcoidosis, patients who become intolerant to infliximab because of an allergic reaction, antibody formation against infliximab, or other serious adverse events can switch to adalimumab, a fully human monoclonal antibody, which was reported effective in one small randomized controlled trial and some observational studies [22-26]. To date, data regarding response to adalimumab in patients previously treated with infliximab are lacking.

The objective of this retrospective observational study was to establish whether adalimumab could achieve improvement or stabilization of the disease in refractory sarcoidosis patients who developed intolerance to infliximab.

METHODS

Study design and study subjects

All refractory sarcoidosis patients referred to the St Antonius Interstitial Lung Diseases Center of Excellence, Nieuwegein, The Netherlands, between January 2008 and April 2015 who were treated with adalimumab after previous treatment with infliximab were examined. The Interstitial Lung Diseases Center of Excellence is a national tertiary referral center for sarcoidosis. Sarcoidosis was diagnosed according to the American Thoracic Society/European Respiratory Society criteria [27]. All patients had biopsy-proven sarcoidosis. Only refractory sarcoidosis patients, who were unresponsive to first- and second-line therapy or who had adverse events from these agents and with signs of disease activity, impaired organ function, or severely diminished quality of life were considered for treatment with TNF-α inhibitors as part of routine patient care. Failure of therapy was determined by a pulmonologist, neurologist, or ophthalmologist, and defined as either clinical or symptomatic progression of disease despite conventional therapy. Medical records were reviewed for demographic data, relevant clinical data, organ involvement, effect of infliximab treatment, and adverse events during the treatment with adalimumab. The study was performed in accordance with the Declaration of Helsinki and its amendments. The study

was approved by the local institutional review board of St Antonius Hospital Nieuwegein, The Netherlands, with registration number LTME/Z-12.33 and acronym ORATS.

Clinical response rate

Response to both TNF-α inhibitors was evaluated. To assess functional response, both the organ of treatment indication and every other organ with documented sarcoid involvement were graded in a systematic manner before the start and after one year of TNF-α inhibitor treatment with infliximab. Additionally, baseline characteristics and the effect of adalimumab after twelve months of treatment, or after six months if evaluation after twelve months was not available, were graded until at least one year after the switch or until discontinuation of adalimumab. We carefully documented whether a patient improved, stabilized or deteriorated.

In case of uveitis, response was classified by an experienced ophthalmologist. In case of hypercalcemia, normalization in serum calcium levels was regarded as improvement. In patients with severely impaired quality of life we used the score on the physical functioning component of the 36-item short form health survey. An increase ≥ 10 points was regarded as improvement, any change < 10 points as stable and a decrease ≤ 10 points as deterioration [28]. In pulmonary sarcoidosis, the change in forced vital capacity (FVC) was used to determine functional response. Improvement of FVC was defined as an increase of FVC of ≥ 5% predicted compared to baseline, as stable when change was < 5% predicted and as deteriorated when it decreased by ≥ 5% predicted [16]. Improvement was scored only when one of the parameters improved without deterioration of the others.

Inflammatory response was classified using the biomarker soluble interleukin-2 receptor (sIL-2R) [29-31]. Improvement was defined as a decrease of the sIL-2R level of ≥ 40% of baseline, as stable when change was < 40% of baseline and as deteriorated when it increased by ≥ 40% [16].

Pulmonary function testing and radiology

Pulmonary function tests were performed using the Master Screen Body system (Jaeger ms-pft analysis unit, Würtzburg, Germany). All chest radiographs were graded at inclusion by a single observer. Radiographic abnormalities were classified in five stages (0 to IV) [27].

Antibodies against infliximab

Antibodies against infliximab were measured using radioimmunoassay by Sanquin, The Netherlands [32]. This assay only detects antibodies against infliximab that are not bound to infliximab. Antibodies against infliximab were measured in patients with clinical loss of response and in patients with severe adverse events or symptoms of an allergic reaction.

Inflammatory serum marker

Quantification of sIL-2R levels (levels > 3000 pg/mL considered elevated) was performed in serum with enzyme immunoassays according to the manufacturer's instructions (until April 2009: Mile-

nia; AMDS Benelux Malden, The Netherlands; 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.

Graphs

Graphs were created using Graphpad Prism 6.01 (Graphpad Software, La Jolla, CA USA).

RESULTS

Study population

Out of the 142 sarcoidosis patients treated with infliximab in our cohort, 18 patients (13%) developed intolerance to infliximab or experienced clinical loss of response together with measurable antibodies against infliximab. No patients with clinical loss of response without measurable antibodies against infliximab switched to adalimumab during the study period (Figure 1).

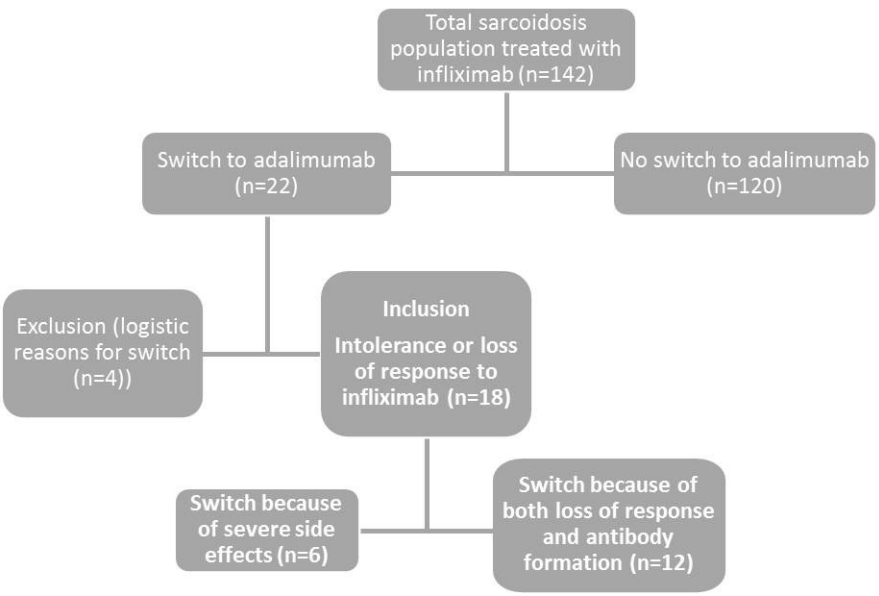


Figure 1. Flow chart of patient inclusion.
Patients initially treated with infliximab who switched to adalimumab were included.

Baseline characteristics of the 18 patients included are summarized in Table 1. Three patients were of African-American ethnicity (#9, 10, 11), all other patients were Caucasians. Most patients (61%, n=11) were former smokers, two patients were current smokers (#1 and 6), and five patients

had never smoked. Previous immunosuppressive therapy consisted of at least three other drugs in 83% (n=15) of patients. All patients were either infliximab responders (n=16), or treatment duration was too short (i.e. less than six months) to achieve response (three and four months after baseline (patient #15 and 6, respectively); n=2). Follow-up after 12 months of treatment was available for 14 patients; for four patients it was only available after six months.

In 15 patients (83%), the initial dose of adalimumab in week 0 was 120 mg, followed by a dose of 80 mg in week 1 and a dose of 40 mg every week thereafter. In three patients, this was 80 mg in week 0 and 40 mg every week thereafter. At the start of adalimumab treatment, most patients (83%) received concomitant immunosuppressive therapy. Eight patients (44%) received MTX (median dose 7.5 mg per week), seven patients (39%) prednisone (median dose 10 mg daily) and one patient (6%) azathioprine (100 mg daily). In most patients (n=13) this concomitant medica-

Table 1. Baseline characteristics of the refractory sarcoidosis patients intolerant to infliximab who switched to adalimumab.

Patient (n=18)	**Age (yrs)**	**Sex**	**Scadding Stage**
Allergic reaction and/or disease progression with presence of antibodies against IFX			
1	36.3	M	II
2	57.8	M	IV
3	42.4	M	IV
4	64.3	F	II
5	61.5	F	II
6	50.7	M	IV
7	65.3	F	IV
8	56.6	F	II
9	31.2	F	II
10	49.0	F	II
11	30.4	F	I
12	40.0	F	II
Allergic reaction or adverse effects without presence of antibodies against IFX			
13	35.2	F	IV
14	44.6	M	0
15	39.4	F	II
16	33.0	M	IV
17	62.6	M	II
18	76.6	M	0
Median (range)	**46.8 (30.4-76.6)**	**8M/10F**	

yrs: years; mo: months; IFX: infliximab; pred: prednisone; MTX: methotrexate; PLAQ: plaquenil; AZA: azathioprine; F: female; M: male.

tion was already part of the patients' regimen to prevent antibody formation. Only in one patient concomitant therapy was changed during adalimumab treatment: patient #3 was also started on azathioprine 200 mg/day after six months, next to prednisone 20 mg/day which he received since the start of the adalimumab treatment. No other additional medication and also no other interventions were initiated during the study period with regard to end points used in this study.

Response

The courses of FVC and of sIL-2R during adalimumab treatment are shown in Figure 2 and Figure 3, respectively. The treatment indications and responses to adalimumab treatment are shown in Table 2. Seven patients (39%) showed improvement of organ function after six or twelve months of therapy, six patients (33%) remained stable and organ function declined in five patients (28%). In two of these latter patients, organ function deteriorated while sIL-2R decreased (Table 3).

	Disease duration (yrs)	Previous immuno-suppressive medication besides IFX	Time interval between stopping IFX and starting ADA (mo)
	10.4	pred, MTX	15.0
	10.9	pred, MTX	15.0
	8.0	pred, MTX	1.3
	2.4	pred, AZA, MTX	0.9
	9.3	pred, MTX, PLAQ	5.1
	8.4	pred, MTX	1.4
	11.2	MTX	1.4
	11.0	pred, MTX, PLAQ	7.0
	3.0	pred, MTX	1.5
	16.7	pred	10.5
	5.5	pred, MTX	8.0
	9.3	pred, PLAQ	2.0
	4.4	pred, MTX	11.2
	5.0	pred, MTX, PLAQ	19.2
	3.8	pred, MTX	11.3
	6.3	pred, MTX	2.1
	4.4	pred, AZA, MTX	2.2
	7.2	pred	15.6
	7.6 (2.4-16.7)		**6.0 (0.9-19.2)**

Among the patients with chest X-ray stage IV (n=6), pulmonary function improved in three patients, was stable in two patients and deteriorated in one patient. Inflammatory biomarker sIL-2R deteriorated in none of the patients, remained stable in seven patients (39%) and improved in 11 patients (61%). In five of the seven patients with stable sIL-2R levels, sIL-2R was reduced by 30 - 40%, which is however just below our cut-off. Median decrease in sIL-2R was 3614 pg/mL. Normalization of sIL-2R (< 3000 pg/mL) occurred in five patients (28%).

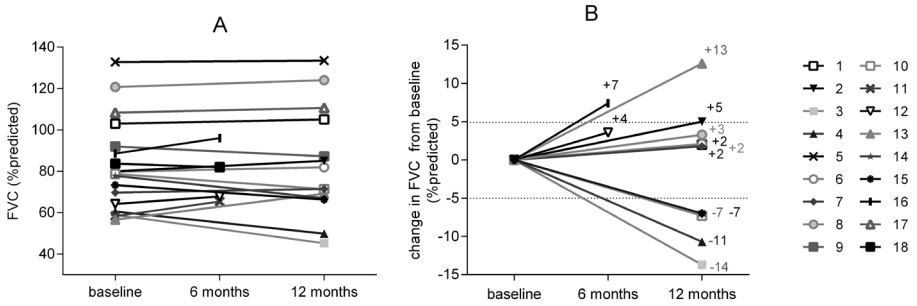


Figure 2. Course of the forced vital capacity (FVC) during adalimumab treatment.
A) In all patients (n=18), B) change compared to baseline in patients with a pulmonary treatment indication (n=12). The dotted lines represent improvement and deterioration of 5% predicted from baseline. Patients #11, 12, 16 and 18 were evaluated after six months, the other patients after twelve months.

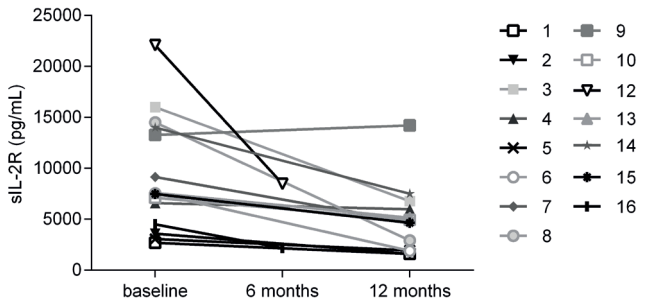


Figure 3. Course of soluble interleukin-2 receptor (sIL-2R) levels during adalimumab treatment.
Data from patients with kidney dysfunction (n=3) are not included; they all improved according to the defined criteria of improvement.

Table 2. Organ function and inflammation response to adalimumab.

Patient	Treatment indication	Organ function (change in FVC after 12 months[a], expressed as % predicted) (stable = change < 5% predicted)	Inflammation (change in sIL-2R after 12 months[a], expressed as pg/mL) (stable = change < 40%)
Allergic reaction and/or disease progression with presence of antibodies against IFX			
1	Pulmonary	S (+2)	S (-39%)
2	Pulmonary	I (+5)	I (-55%)
3	Pulmonary	D (-14)	I (-58%)
4	Pulmonary	D (-11)	S (-9%)
5	Severely impaired QoL	S	S (-37%)
6	Pulmonary	S (+2)	I (-75%)
7	Pulmonary	S (+2)	I (-48%)
8	Pulmonary	S (+3)	I (-80%)
9	Uveitis	I	S (+7%)
10	Pulmonary	D (-7)	S (-30%)
11	Hypercalcemia	I	I (-80%)[b]
12	Pulmonary	S (+4)	I (-62%)
Allergic reaction or adverse effects without presence of antibodies against IFX			
13	Pulmonary	I (+13)	S (-32%)
14	Severely impaired QoL	I	I (-46%)
15	Pulmonary	D (-7)	S (-38%)
16	Pulmonary	I (+7)	I (-52%)
17	Hypercalcemia	I	I (-80%)[b]
18	Uveitis	D	I (-51%)[b]

[a] patients #11, 12, 16 and 18 were evaluated after six months of treatment.
[b] patients with kidney dysfunction.
FVC: forced vital capacity; sIL-2R: soluble interleukin-2 receptor; IFX: infliximab; QoL: quality of life; I: improved; S: stable; D: deteriorated.

Table 3. Cross table of response with regard to organ function and inflammation.

			Organ function	
Inflammation	Improved	5	4	2
	Stable	2	2	3
	Deteriorated	0	0	0

Adverse events and treatment discontinuation

Severe adverse events occurred in four patients (22%). One of these patients discontinued adalimumab due to a lupus-like reaction. Most reported adverse events in this study were infections, with three severe and seven mild infections in seven patients (39% of patients). Seven patients reported no adverse events. Adverse events are described in Table 4.

Table 4. Reported adverse events during treatment with adalimumab.

Adverse event	n
Severe adverse events	
Neutropenia and candidiasis requiring ICU admittance	1
Pneumonia requiring hospitalization, antibiotic treatment, and temporarily discontinuation of adalimumab	2
Lupus-like reaction (discontinuation of treatment)	1
Other adverse events	
Mild infection	7 (in 5 patients)
Injection site reaction	2
Headache	2
Nausea	2
Pyrosis	1
Rash	1
Arthritis	1
Edema	1
Weight loss	1

DISCUSSION

This study found clinical improvement in 39% and stabilization in 33% of refractory sarcoidosis patients treated with adalimumab after becoming intolerant to infliximab. Inflammation, measured by change in sIL-2R, did not increase in any of the patients treated with adalimumab.

Our response rate is in line with the findings of Baughman *et al.* [33], who reported that 48.1% of patients improved, 14.8% was stable and 37.0% worsened on adalimumab treatment. Baughman *et al.* [34] also postulated adalimumab as an alternative to infliximab, especially for patients who develop significant allergic reactions to infliximab. Our study is the first to provide data to support this statement. Previous observational and randomized controlled studies have provided evidence for effectiveness of adalimumab and infliximab in sarcoidosis [13,16,22,25]. No study has compared adalimumab with infliximab for sarcoidosis. Our study design was inappropriate to perform such a comparison, which would require a randomized study. However, in view of the low numbers of patients requiring third-line therapy in sarcoidosis, pharmaceutical companies are not interested in acquiring market authorization of the drug for sarcoidosis and will not invest in these studies. As a result, current clinical practice, with infliximab as the most often used third-line agent, will likely not change. This makes the results of this study especially relevant, as they show that adalimumab is a viable option for patients intolerant to infliximab.

Inflammatory biomarker and organ response

We describe a decrease of > 40% of inflammatory biomarker sIL-2R in 61% of patients and additionally a decrease of 30 - 40% in 28% of patients. Median decrease in sIL-2R was 3614 pg/mL. Since high levels of sIL-2R have been associated with worse long-term clinical outcome, these findings are promising [11]. A previous study on the effect of MTX on sIL-2R in sarcoidosis showed a correlation between change in sIL-2R and lung function improvement [35].

With regards to organ function, most patients in this study improved (39%, n=7) and, additionally, six patients (33%) remained stable. The main objective of initiating treatment with adalimumab after the development of intolerance to infliximab was to achieve stabilization or improvement of the disease. Currently, for these patients, adalimumab is the only alternative anti-TNF agent, since no other agents specifically targeted at TNF with known efficacy in sarcoidosis are available: in sarcoidosis two other anti-TNF agents have been studied, etanercept and golimumab, but were found to be insufficiently effective [36,37].

Two patients improved in terms of inflammatory activity measured by sIL-2R, while their organ function decreased. One of these patients (#3) suffered from pneumonia, explaining the deterioration in pulmonary function. In the other patient, no other cause of the deterioration of organ function was found.

Adalimumab, being an anti-inflammatory drug, is not expected to affect pulmonary fibrosis. Mostard *et al.* [38] found a high inflammatory activity on ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) scan in patients with fibrotic changes on high-resolution computed tomography (HRCT) scan, assuming that anti-TNF-α treatment is an option even in these severe cases with fibrosis. In several other studies patients with stage IV pulmonary sarcoidosis responded to anti-TNF-α treatment with infliximab [13,15,16]. In our study in five out of the six patients (83%) with fibrotic changes (stage IV) FVC indeed improved (n=3) or remained stable (n=2).

Concomitant use of MTX has been reported to reduce immunogenicity in adalimumab treatment in rheumatoid arthritis. A study by Krieckaert *et al.* [39], showed that patients using MTX less often developed antibodies against adalimumab. Another study showed that concomitant use of MTX plays an important role in pharmacokinetics of adalimumab in rheumatoid arthritis. They found that the median adalimumab concentration in patients who received concomitant MTX was higher compared to patients who received no concomitant MTX (7.4 µg/mL versus 4.1 µg/mL, respectively). Moreover, in the group of patients who received concomitant MTX, more patients were good responders. The median MTX dose in these good responders was 25 mg/week [40]. In our population a lower dose of MTX was used (median 7.5 mg/week). This might explain why we did not find a beneficial effect of concomitant MTX use in our population (data not shown). Moreover, the sample size of this study was rather small.

Three patients had kidney dysfunction and associated high levels of sIL-2R, as previously described [41]. Their kidney function did not change during therapy. In all three patients, sIL-2R decreased by > 40%.

Adverse events

Most reported adverse events in this study were infections. Other studies have shown variable rates of infection during adalimumab treatment, ranging from none [24,42] to multiple infections (five infections in ten treated patients) [25], similar to what was found in our study. Interestingly, the infection rate during adalimumab treatment in our population was higher than the rate during infliximab treatment described recently (13 infections in 56 sarcoidosis patients (23%) over a period of six months) [16]. A possible explanation for this difference between adalimumab and infliximab could be that no standardized control of laboratory signs of infection (CRP, leucocyte count) was performed before each administration of adalimumab, whereas during treatment with infliximab, CRP and leucocyte count are determined before every infliximab infusion. In case of increased inflammatory parameters, infliximab infusion is often postponed and antibiotic prophylaxis is given.

One patient had a lupus-like reaction after the dose was increased to 80 mg/week. The skin lesions disappeared after treatment was discontinued. Lupus-like reaction to anti-TNF-α therapy has been described before in other treatment indications, most often with etanercept and infliximab, but also with adalimumab. The pathogenic mechanism behind this adverse event remains unknown and no knowledge on the relation with the dosage is available [43].

Limitations of the present study are the small number of patients and the retrospective study design. In sarcoidosis, different adalimumab dosing regimens have been described (most often 40 mg every other week and 40 mg/week) [22-26]. In our study population in most patients an induction regimen of 120 mg at week 0 and 80 mg at week 1 was used. This regimen is comparable to the regimen studied and approved of by the FDA and EMA (160 mg at week 0, 80 mg at week 2) [44]. The maintenance regimen used in our study was high, 40 mg every week, compared to other diseases such as Crohn's disease. This high maintenance regimen has been studied in sarcoidosis before [24] and a study using the Delphi method showed that most experts in the field recommend this maintenance dosing regimen [17]. This regimen is also in line with maintenance dosing regimen of infliximab that is used in sarcoidosis: infliximab is also dosed in a higher fashion in sarcoidosis (5 mg/kg every 4 weeks) compared to Crohn's disease (5 mg/kg every 8 weeks). Interestingly, normalization of sIL-2R (< 3000 pg/mL) was only achieved in 28% of patients. Perhaps the maintenance dose of 40 mg/week was insufficient or treatment duration was too short. Therefore, future studies should focus on finding the optimal dosing regimen of adalimumab in sarcoidosis.

CONCLUSIONS

Adalimumab is an effective alternative for sarcoidosis patients intolerant to infliximab. The switch to adalimumab achieved clinical improvement in 39% and stabilization in 33% of patients intolerant to infliximab. Further research is needed to develop guidelines on how to use adalimumab to treat sarcoidosis, in terms of dosing regimen.

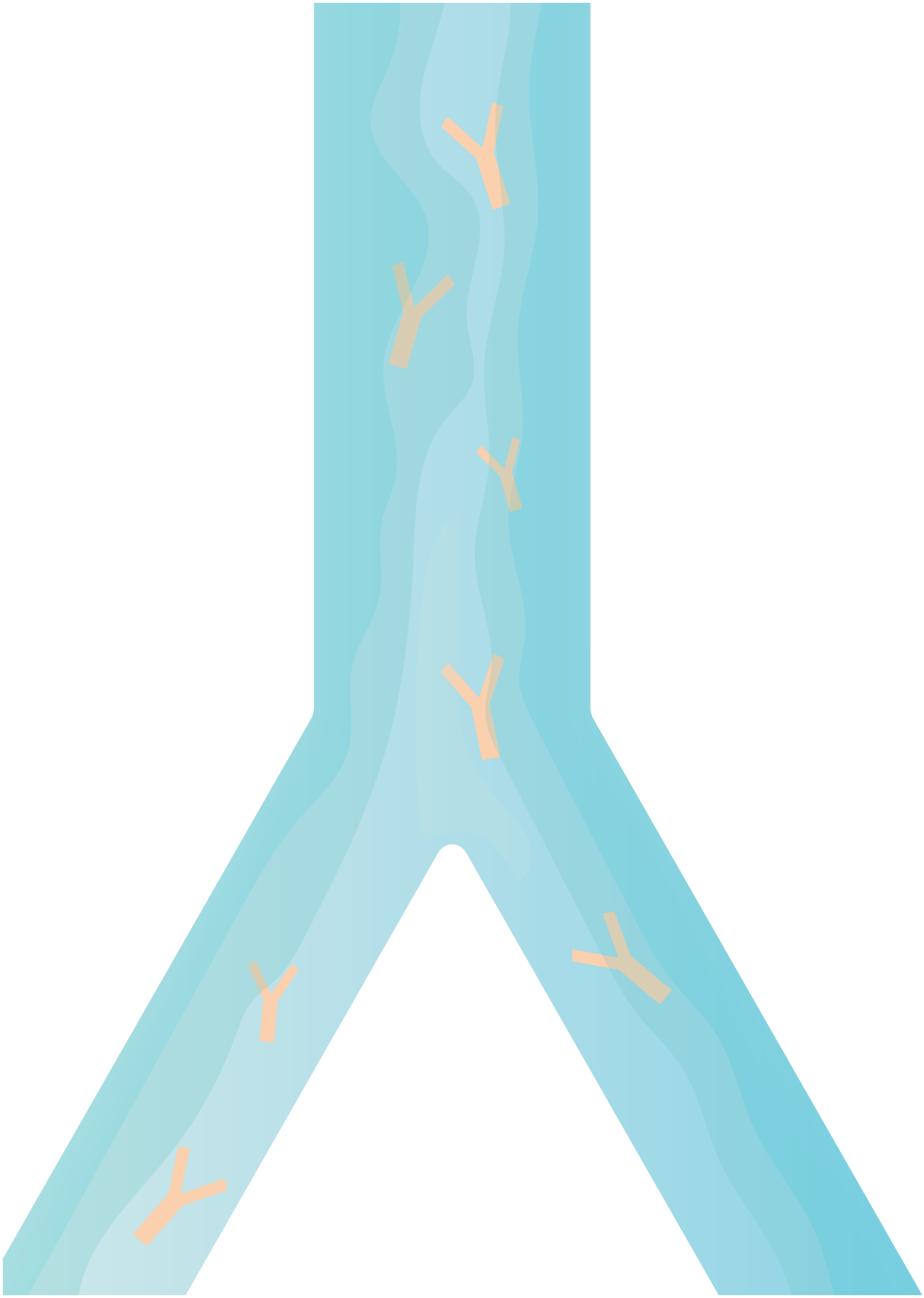
REFERENCES

1. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. *Lancet* 2013; 383: 1155-1167.
2. Grutters JC, van den Bosch JM. Corticosteroid treatment in sarcoidosis. *Eur Respir J* 2006; 28: 627-636.
3. Paramothayan NS, Lasserson TJ, Jones PW. Corticosteroids for pulmonary sarcoidosis. *Cochrane Database Syst Rev* 2005; (2): CD001114.
4. Vorselaars AD, van Moorsel CH, Deneer VH, Grutters JC. Current therapy in sarcoidosis, the role of existing drugs and future medicine. *Inflamm Allergy Drug Targets* 2013; 12: 369-377.
5. Baughman RP, Winget DB, Lower EE. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. *Sarcoidosis Vasc Diffuse Lung Dis* 2000; 17: 60-66.
6. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. *Arch Intern Med* 1995; 155: 846-851.
7. Cremers JP, Drent M, Bast A, Shigemitsu H, Baughman RP, Valeyre D, Sweiss NJ, Jansen TL. Multinational evidence-based World Association of Sarcoidosis and Other Granulomatous Disorders recommendations for the use of methotrexate in sarcoidosis: integrating systematic literature research and expert opinion of sarcoidologists worldwide. *Curr Opin Pulm Med* 2013; 19: 545-561.
8. Vorselaars AD, Wuyts WA, Vorselaars VM, Zanen P, Deneer VH, Veltkamp M, Thomeer M, van Moorsel CH, Grutters JC. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* 2013; 144: 805-812.
9. Judson MA. The treatment of pulmonary sarcoidosis. *Respir Med* 2012; 106: 1351-1361.
10. Baughman RP, Lower EE, Drent M. Inhibitors of tumor necrosis factor (TNF) in sarcoidosis: who, what, and how to use them. *Sarcoidosis Vasc Diffuse Lung Dis* 2008; 25: 76-89.
11. Ziegenhagen MW, Benner UK, Zissel G, Zabel P, Schlaak M, Muller-Quernheim J. Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am J Respir Crit Care Med* 1997; 156: 1586-1592.
12. Morgenthau AS, Iannuzzi MC. Recent advances in sarcoidosis. *Chest* 2011; 139: 174-182.
13. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
14. Hostettler KE, Studler U, Tamm M, Brutsche MH. Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218-224.
15. van Rijswijk HNAJ, Vorselaars ADM, Ruven HTJ, Keijsers RGM, Zanen P, Korenromp IHE, Grutters JC. Changes in disease activity, lung function and quality of life in patients with refractory sarcoidosis after anti-TNF treatment. *Expert Opinion on Orphan Drugs* 2013; 1: 437-443.
16. Vorselaars AD, Crommelin HA, Deneer VH, Meek B, Claessen AM, Keijsers RG, van Moorsel CH, Grutters JC. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J* 2015; 46: 175-185.
17. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
18. Colombel JF, Feagan BG, Sandborn WJ, Van Assche G, Robinson AM. Therapeutic drug monitoring of biologics for inflammatory bowel disease. *Inflamm Bowel Dis* 2012; 18: 349-358.
19. Ordas I, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2012; 10: 1079-1087.
20. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Severe infusion reactions to infliximab: aetiology, immunogenicity and risk factors in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 34: 51-58.
21. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. *Am J Gastroenterol* 2013; 108: 40-47.
22. Sweiss NJ, Noth I, Mirsaeidi M, Zhang W, Naureckas ET, Hogarth DK, Strek M, Caligiuri P, Machado RF, Niewold TB,

Garcia JG, Pangan AL, Baughman RP. Efficacy Results of a 52-week Trial of Adalimumab in the Treatment of Refractory Sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 46-54.

23. Milman N, Graudal N, Loft A, Mortensen J, Larsen J, Baslund B. Effect of the TNF-alpha inhibitor adalimumab in patients with recalcitrant sarcoidosis: a prospective observational study using FDG-PET. *Clin Respir J* 2012; 6: 238-247.
24. Erckens RJ, Mostard RL, Wijnen PA, Schouten JS, Drent M. Adalimumab successful in sarcoidosis patients with refractory chronic non-infectious uveitis. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 713-720.
25. Pariser RJ, Paul J, Hirano S, Torosky C, Smith M. A double-blind, randomized, placebo-controlled trial of adalimumab in the treatment of cutaneous sarcoidosis. *J Am Acad Dermatol* 2013; 68: 765-773.
26. Minnis PA, Poland M, Keane MP, Donnelly SC. Adalimumab for refractory pulmonary sarcoidosis. *Ir J Med Sci* 2015; 185: 969-971.
27. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
28. Swigris JJ, Brown KK, Behr J, du Bois RM, King TE, Raghu G, Wamboldt FS. The SF-36 and SGRQ: validity and first look at minimum important differences in IPF. *Respir Med* 2010; 104: 296-304.
29. Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R, Irifune K, Higaki J. Comparative evaluation of serum markers in pulmonary sarcoidosis. *Chest* 2010; 137: 1391-1397.
30. Keicho N, Kitamura K, Takaku F, Yotsumoto H. Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis. *Chest* 1990; 98: 1125-1129.
31. Rothkrantz-Kos S, van Dieijen-Visser MP, Mulder PG, Drent M. Potential usefulness of inflammatory markers to monitor respiratory functional impairment in sarcoidosis. *Clin Chem* 2003; 49: 1510-1517.
32. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 711-715.
33. Baughman RP. Tumor necrosis factor inhibition in treating sarcoidosis: the American experience. *Revista Portuguesa de Pneumonologia* 2007; 13: S47-S50.
34. Baughman RP, Lower EE, Kaufman AH. Ocular sarcoidosis. *Semin Respir Crit Care Med* 2010; 31: 452-462.
35. Vorselaars AD, van Moorsel CH, Zanen P, Ruven HJ, Claessen AM, van Velzen-Blad H, Grutters JC. ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. *Respir Med* 2015; 109: 279-285.
36. Judson MA, Baughman RP, Costabel U, Drent M, Gibson KF, Raghu G, Shigemitsu H, Barney JB, Culver DA, Hamzeh NY, Wijsenbeek MS, Albera C, Huizar I, Agarwal P, Brodmerkel C, Watt R, Barnathan ES. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. *Eur Respir J* 2014; 44: 1296-1307.
37. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest* 2003; 124: 177-185.
38. Mostard RL, Voo S, van Kroonenburgh MJ, Verschakelen JA, Wijnen PA, Nelemans PJ, Erckens RJ, Drent M. Inflammatory activity assessment by F18 FDG-PET/CT in persistent symptomatic sarcoidosis. *Respir Med* 2011; 105: 1917-1924.
39. Krieckaert CL, Nurmohamed MT, Wolbink GJ. Methotrexate reduces immunogenicity in adalimumab treated rheumatoid arthritis patients in a dose dependent manner. *Ann Rheum Dis* 2012; 71: 1914-1915.
40. Pouw MF, Krieckaert CL, Nurmohamed MT, van der Kleij D, Aarden L, Rispens T, Wolbink G. Key findings towards optimising adalimumab treatment: the concentration-effect curve. *Ann Rheum Dis* 2015; 74: 513-518.
41. Verwoerd A, Vorselaars AD, van Moorsel CH, Bos WJ, van Velzen-Blad H, Grutters JC. Discrepant elevation of sIL-2R levels in sarcoidosis patients with renal insufficiency. *Eur Respir J* 2015; 46: 277-280.
42. Riancho-Zarrabeitia L, Calvo-Rio V, Blanco R, Mesquida M, Adan AM, Herreras JM, Aparicio A, Peiteado-Lopez D, Cordero-Coma M, Garcia Serrano JL, Ortego-Centeno N, Maiz O, Blanco A, Sanchez-Burson J, Gonzalez-Suarez S, Fonollosa A, Santos-Gomez M, Gonzalez-Vela C, Loricera J, Pina T, Gonzalez-Gay MA. Anti-TNF-alpha therapy in refractory uveitis associated with sarcoidosis: Multicenter study of 17 patients. *Semin Arthritis Rheum* 2015; 45: 361-368.

43. Amarante CF, Acedo LM, Rabay FM, Campos Bdo E, Lira ML, Mandelbaum SH. Drug-induced lupus with leukocytoclastic vasculitis: a rare expression associated with adalimumab. *An Bras Dermatol* 2015; 90: 121-124.
44. Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Colombel JF, Panaccione R, D'Haens G, Li J, Rosenfeld MR, Kent JD, Pollack PF. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. *Ann Intern Med* 2007; 146: 829-838.



6

Genetic variants in *TNFRSF1A*, *TNFRSF1B* and *HLA-DRB1* are associated with response parameters of infliximab in severe sarcoidosis

Heleen A Crommelin
Joanne J van der Vis
Adriane DM Vorselaars
Vera HM Deneer
Jan C Grutters
Coline HM van Moorsel

ABSTRACT

Background: Sarcoidosis is a systemic, granulomatous disease with a variable and sometimes chronic, progressive clinical course. The anti-tumor necrosis factor (TNF) agent infliximab can be an effective treatment option in severe sarcoidosis, but response is highly variable. In autoimmune diseases, genetic variation in TNF, TNF receptors, Fcγ-receptors and HLA has been associated with response to infliximab therapy. We tested whether genetic variations associated with change in clinical response parameters in a cohort of severe sarcoidosis patients treated with infliximab.

Methods: Patients (n=106) were genotyped for *TNFRSF1A* rs1800693*, TNFRSF1B* T196G*, FCGR2A* G131A*, FCGR3A* C-158A and *HLA* tag SNPs rs2040410A and rs3135388T to capture HLA-DRB1*0301 and HLA-DRB1*1501, respectively. Pulmonary function tests and inflammatory biomarkers were evaluated at baseline and after six months of treatment. Change from baseline of pulmonary function tests and inflammatory biomarkers soluble interleukin-2 receptor (sIL-2R) and angiotensin-converting enzyme (ACE) were compared between carriers and non-carriers of a genetic variation.

Results: The mean improvement in the diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (DLCOc) was lower in carriers of the *TNFRSF1A* rs1800693 G allele (GG+GA) than in non-carriers (AA) (0.5 vs 6.1% predicted, p=0.001). Furthermore, reduction in sIL-2R and ACE was lower in *TNFRSF1A* rs1800693 G allele carriers than in non-carriers (2473 vs 6273 pg/mL, p=0.004 and 16 vs 38 U/L, p=0.03, respectively). Regarding *TNFRSF1B* T196G, carriers of the T allele had a mean decrease in ACE of 25 U/L versus an increase of 15 U/L in non-carriers of the T allele (p=0.02). Finally, HLA-DRB*0301 negative patients (tag SNP rs2040410 GG) showed a significantly higher reduction in sIL-2R compared to HLA-DRB1*0301 positive patients (tag SNP rs2040410 carriers of the A allele) (3996 versus 2283 pg/mL, p=0.03).

Conclusions: Genetic variants in the genes *TNFRSF1A* rs1800693, *TNFRSF1B* T196G and HLA-DRB1*0301 tag SNP associate with changes in clinical and inflammatory parameters in patients with severe sarcoidosis patients treated with infliximab. These findings require further confirmation in other studies.

INTRODUCTION

Sarcoidosis is a systemic, granulomatous disease with a variable and sometimes chronic, progressive clinical course. Virtually any organ can be affected, but pulmonary involvement is most common and found in approximately 95% of the patients. Although the etiology of sarcoidosis remains unknown, tumor necrosis factor (TNF) has been found to play an important role in granuloma formation. Macrophages in bronchoalveolar lavage of patients with sarcoidosis excrete higher amounts of TNF compared to healthy controls. Additionally, patients with a high level of TNF release are at greater risk of disease progression compared to patients with normal TNF release [1]. Systemic therapy is indicated in patients in whom organ function is threatened by the disease. In patients who are refractory to both corticosteroids and second-line therapeutic agents, such as methotrexate or azathioprine, agents specifically targeting TNF are an effective third-line treatment option [2]. Multiple agents specifically targeting TNF are available, but in sarcoidosis the anti-TNF agent that has been most extensively studied is infliximab. Unfortunately, not all patients respond to infliximab therapy and treatment is not only costly but can also potentially have side effects [3]. Therefore, finding markers that predict the response to infliximab is of the utmost importance. In sarcoidosis, patients with a higher inflammatory activity as measured by ^{18}F-fluordeoxyglucose by positron emission tomography (^{18}F-FDG PET) of the pulmonary parenchyma were found to have a significantly larger improvement in pulmonary function during infliximab treatment compared to patients with lower inflammatory activity [4].

Genetics has also been postulated as a possible factor influencing response to biologicals specifically targeting TNF. In sarcoidosis the only study that has been performed on pharmacogenetics reported an association between the *TNF* gene situated in the HLA class II region and response. Response rate to infliximab or adalimumab was higher in patients without the minor allele A at *TNF* -308 compared with patients carrying the A allele [5]. However, this finding has not been investigated in another study and therefore has not been replicated.

Several other genetic variations have been associated with the response to anti-TNF agents in other immune-mediated inflammatory diseases, but these genetic variations have not been investigated for sarcoidosis [6-10]. The aim of this study was to test whether genetic variations in TNF, TNF receptors (TNFR), i.e. TNFR1 and TNFR2, and Fcγ-receptors that have been studied in other immune-mediated inflammatory diseases or, in the case of TNFR1, are hypothesized to influence the response to infliximab because of functional impact are associated with change in clinical response parameters in a cohort of severe sarcoidosis patients treated with infliximab. We also tested two genetic variants in *HLA-DRB1* that have been linked with two different phenotypes of sarcoidosis.

METHODS

Study population

All sarcoidosis patients treated with infliximab at St Antonius Hospital Interstitial Lung Diseases Center of Excellence, Nieuwegein, The Netherlands, between March 2005 and March 2016 were included. St Antonius Hospital is a national tertiary referral center 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 infliximab treatment. The diagnosis was made according to the American Thoracic Society/European Respiratory Society criteria [11]. The treating physician judged disease severity at the moment of initiation based on loss of function (*e.g.* lung function or cardiac function), impaired quality of life and disease activity on ^{18}F-FDG PET scan. Exclusion criteria for infliximab treatment were vaccination with live or bacterial vaccines within the previous three months, active or untreated latent tuberculosis, serious infections in the last two months, heart failure unrelated to sarcoidosis, active hepatitis, history of allergic reactions to monoclonal antibodies and their fragments, opportunistic infections within the last six months, HIV, transplantation, known malignancy, pregnancy or breastfeeding. Additional exclusion criteria were treatment duration with infliximab for less than six months, no DNA available, treatment with Inflectra® or previous anti-TNF treatment at the Academic Hospital of Maastricht.

Patients were part of a study cohort that has been described previously [4] or part of the Interstitial Lung Disease Biobank Study of the St Antonius Hospital Interstitial Lung Diseases Center of Excellence, Nieuwegein, The Netherlands. Protocols were approved by the institutional medical ethics research committee of the St Antonius Hospital Nieuwegein (registration number R-10.13A and R05-08A, respectively). All patients provided written informed consent.

Patients received infliximab (Remicade®) 5 mg/kg at week 0, 2 and every four weeks or every six weeks thereafter. Patients who started treatment before 2009 were treated with a dosing interval of six weeks and other patients with a dosing interval of four weeks. Evaluation of the treatment response was performed after six months, including pulmonary function and inflammatory biomarkers.

Response was also scored as improved, stable or deteriorated after one year of treatment in patients who continued treatment for one year or more as previously described by Wijnen *et al.* [5].

Evaluation of treatment response

Pulmonary function was evaluated by measuring forced vital capacity (FVC), forced expiratory volume in one second (FEV1) and diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (DLCOc). Pulmonary function tests were performed using the Master Screen Body system (Jaeger ms-pft analysis unit, Würtzburg, Germany). Pulmonary involvement was defined as pulmonary parenchymal abnormalities on high resolution computed tomography (HRCT) scan.

The inflammatory biomarkers soluble interleukin-2 receptor (sIL-2R) and angiotensin-converting enzyme (ACE) were used to evaluate inflammatory disease activity. Quantification of sIL-2R levels was performed in serum with enzyme immunoassays according to the manufacturer's instructions (until April 2009: Milenia; AMDS Benelux Malden, The Netherlands; 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. Levels of sIL-2R > 3000 pg/mL were considered elevated. Angiotensin-converting enzyme (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).

For comparison with the previously reported association between *TNF* -308 and response [5], the change in organ function of all organs involved was evaluated after one year. The patient was scored as improved if the function of the organ that was the major indication for treatment showed an increase of 10% or more without deterioration of other organs involved. The patient was scored as stable if organ function did not change with 10% or more and no other organs involved deteriorated. The patient had deteriorated if any of the organs involved deteriorated by 10% or more in organ function. Pulmonary involvement was evaluated by FVC, FEV1 and DLCOc. Uveitis was evaluated by an experienced ophthalmologist and small fiber neuropathy was evaluated using the Small Fiber Neuropathy Screening List (SFNSL) and fatigue by CIS score [12]. The inflammatory biomarkers sIL-2R and ACE were also taken into account.

Study data were collected and managed using the Research Electronic Data Capture (REDCap) tools hosted at St Antonius Hospital. The REDCap is a secure, web-based application designed to support data capture for research studies [13].

Genotyping

Genomic DNA was extracted from peripheral blood. Taqman single nucleotide polymorphism (SNP) genotyping assays and an ABI 7500Fast analyzer (Applied Biosystems, Foster City, CA) were used to genotype *TNF* G-308A (rs1800629), *FCGR2A* G131A (rs1801274), *FCGR3A* C-158A (rs396991), *TNFRSF1A* (rs1800693), *TNFRSF1B* T196G (rs1061622) and rs3135388. Rs2040410 was genotyped by a restriction fragment length polymorphism (RFLP) assay. Tag SNP rs2040410A and rs3135388T were used to capture HLA-DRB1*0301 and HLA-DRB1*1501, respectively [14].

Statistical analysis

Continuous variables were expressed as mean ± SD. Categorical variables were expressed as frequencies and percentages. Chi-square table was used to compare the observed number of each genotype with the expected number for a population in Hardy-Weinberg equilibrium ($p > 0.05$). Independent-samples t-test was used to compare change from baseline and baseline values of pulmonary function tests and inflammatory biomarkers between carriers and non-carriers of a genetic variation. Logistic regression was conducted to test the association between genetic variation *TNF* G-308A and response (improved versus stable or deteriorated). Statistical

analysis was performed using SPSS for Windows (version 21.0; IBM, Armonk, NY, USA). Graphs were created using GraphPad Prism 7.02 (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered significant.

RESULTS

A total of 157 patients were treated with infliximab between March 2005 and March 2016. In 18 patients the biosimilar Inflectra® was initiated, in three patients infliximab treatment was initiated in another hospital. DNA was not available in 13 patients and one patient had been included in the Wijnen study [5] and was therefore excluded. In 16 patients treatment was discontinued before completion of six months because of adverse events (n=12), disease progression (n=2) and undisclosed reasons (n=2). Ultimately, 106 patients treated with Remicade® were found eligible and were included in this study. Baseline pulmonary function tests were impaired, especially DLCOc, and inflammatory biomarkers sIL-2R and ACE were high (Table 1). All genetic variants were in Hardy-Weinberg equilibrium (Table 2).

Genotype and change in parameters after six months of treatment

In patients with *TNFRSF1A* rs1800693 AA genotype mean improvement in DLCOc after six months of treatment was significantly higher than in carriers of the G allele carrier (AG+GG) (6.1 vs 0.5% predicted, p=0.001). In the subgroup of patients with pulmonary involvement (n=87) similar differences were observed (6.2 vs 1.6% predicted, p=0.008, Figure 1,Table 3). In the total population of patients, patients with the *TNFRSF1A* rs1800693 AA genotype showed a larger mean decrease in both sIL-2R (6273 vs 2473 pg/mL, p=0.004) and ACE (38 vs 16 U/L, p=0.03), compared with carriers of the G allele (AG+GG).

Table 1. Baseline characteristics of the study subjects.

Characteristic	All patients
Subjects, n	106
Male	65 (62)
Age, years	48.5 ± 9.2
Disease duration at initiation of infliximab, years	5.9 ± 6.0
Diagnosis of sarcoidosis	
Biopsy	98 (93)
Bronchoalveolar lavage	6 (6)
Clinical	2 (2)
Previous medication	
Prednisone	99 (93)
Methotrexate	87 (82)
Azathioprine	8 (8)
Leflunomide	2 (2)
Hydroxychloroquine	13 (12)
Infliximab	17 (16)
Adalimumab	3 (3)
Golimumab	2 (2)
Etanercept	1 (1)
Mycophenolate mofetil	1 (1)
Pulmonary function tests	
FVC, % predicted	81.7 ± 19.1
FEV1, % predicted	71.3 ± 23.0
DLCOc, % predicted	64.9 ± 18.5
Pulmonary involvement	87 (82)
Inflammatory biomarkers	
ACE, U/L	82.2 ± 45.2
sIL-2R, pg/mL	7156 ± 6706
Interval	
4 weeks	85 (80)
6 weeks	21 (20)
Co-medication	
Prednisone	26 (25)
Methotrexate	79 (75)
Azathioprine	3 (3)
Leflunomide	2 (2)
Hydroxychloroquine	2 (2)
None	7 (7)

Data are presented as n (%) or mean ± SD, unless stated otherwise. FVC: forced vital capacity; FEV1: forced expiratory volume in one second; DLCOc: diffusing capacity for carbon monoxide corrected for hemoglobin; ACE: angiotensin-converting enzyme; sIL-2R: soluble interleukin-2 receptor.

Table 2. Genotype frequencies.

SNP (allele) dbSNP	Genotype	Frequency, n (%)	Hardy-Weinberg equilibrium
TNF G-308A	GG	78 (74)	0.07
	GA	23 (22)	
	AA	5 (5)	
FCGR2A G131A	GG	27 (26)	0.22
	GA	59 (56)	
	AA	20 (19)	
FCGR3A C-158A	CC	13 (12)	0.53
	CA	44 (42)	
	AA	49 (46)	
TNFRSF1A rs1800693	AA	32 (30)	0.60
	AG	50 (47)	
	GG	24 (23)	
TNFRSF1B T196G	TT	68 (64)	0.70
	TG	33 (31)	
	GG	5 (5)	
rs2040410	GG	88 (83)	0.86
(A tags HLA-DRB*0301)	GA	17 (16)	
	AA	1 (1)	
rs3135388	CC	69 (65)	0.30
(T tags HLA-DRB*1501)	CT	35 (33)	
	TT	2 (2)	

Table 3. Overview of genotype associated with the response to infliximab in severe sarcoidosis.

Gene	SNP/rs number	Genotype associated with higher response to infliximab	Genotype associated with lower response to infliximab	Change in response parameter	p-value	Mean change in response parameter
In all patients (n=106)						
TNFRSF1A	rs1800693	AA	AG+GG	DLCOc	0.001	+6.1 vs +0.5% predicted
TNFRSF1A	rs1800693	AA	AG+GG	sIL-2R	0.004	-6273 vs -2473 pg/mL
TNFRSF1A	rs1800693	AA	AG+GG	ACE	0.03	-38 vs -16 U/L
TNFRSF1B	T196G	TT+TG	GG	ACE	0.02	-25 vs +15 U/L
HLA-DRB1*0301	rs2040410	GG	GA+AA	sIL-2R	0.03	-3996 vs -2283 pg/mL
In patients with pulmonary involvement according to HRCT (n=87)						
TNFRSF1A	rs1800693	AA	AG+GG	DLCOc	0.008	+6.2 vs +1.6% predicted

SNP: single nucleotide polymorphism; DLCOc: diffusing capacity of the lung for carbon monoxide corrected for hemoglobin; ACE: angiotensin-converting enzyme; sIL-2R: soluble interleukin-2 receptor.

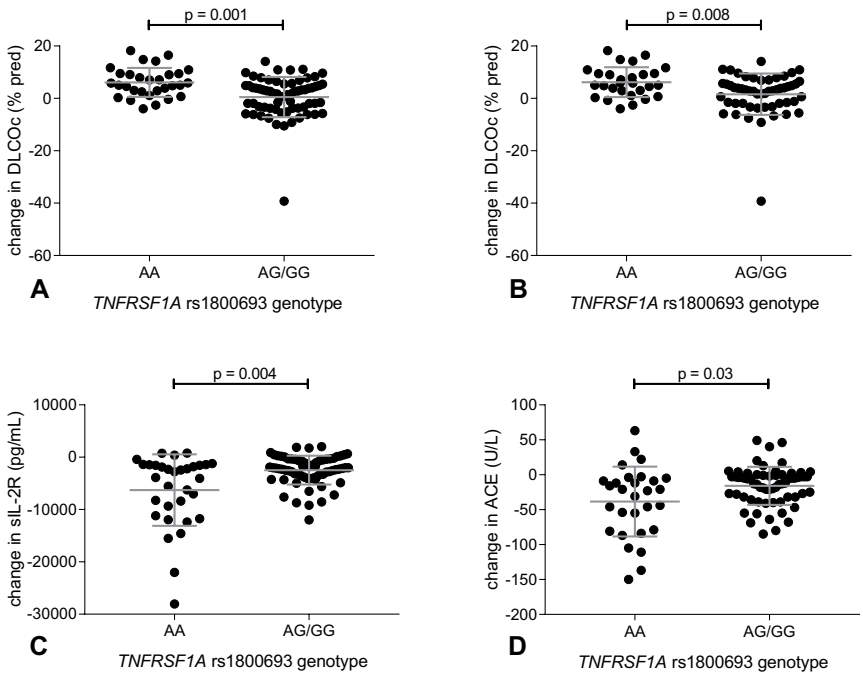


Figure 1. Change in DLCOc, sIL-2R and ACE according to *TNFRSF1A* rs1800693 genotype after six months of infliximab treatment.
A) change in DLCOc in all patients, B) change in DLCOc in patients with pulmonary involvement, C) change in sIL-2R in all patients, D) change in ACE in all patients. DLCOc: diffusing capacity of the lung for carbon monoxide corrected for hemoglobin; % pred: % predicted; sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme. Grey bars represent mean ± SD.

Baseline DLCOc, sIL-2R and ACE were significantly different between patients with the *TNFRSF1A* rs1800639 AA genotype compared to carriers of the G allele: the mean baseline DLCOc was significantly lower in patients with the *TNFRSF1A* rs1800693 AA genotype compared with carriers of the G allele (AG+GG) (59.0 vs 67.5% predicted, p=0.04). The mean baseline values of sIL-2R and ACE were higher in patients with the *TNFRSF1A* rs1800693 AA genotype compared to carriers of the G allele (AG+GG) (sIL-2R 10529 vs 5545 pg/mL, p=0.007 and ACE 103.8 vs 72.3 U/L, p=0.008). In the subgroup of patients with pulmonary involvement (n=87) the mean baseline DLCOc was lower in patients with the AA genotype compared to G allele carrier patients, but this difference was not statistically significant (58.3 vs 63.3% predicted, p=0.21, Figure 2).

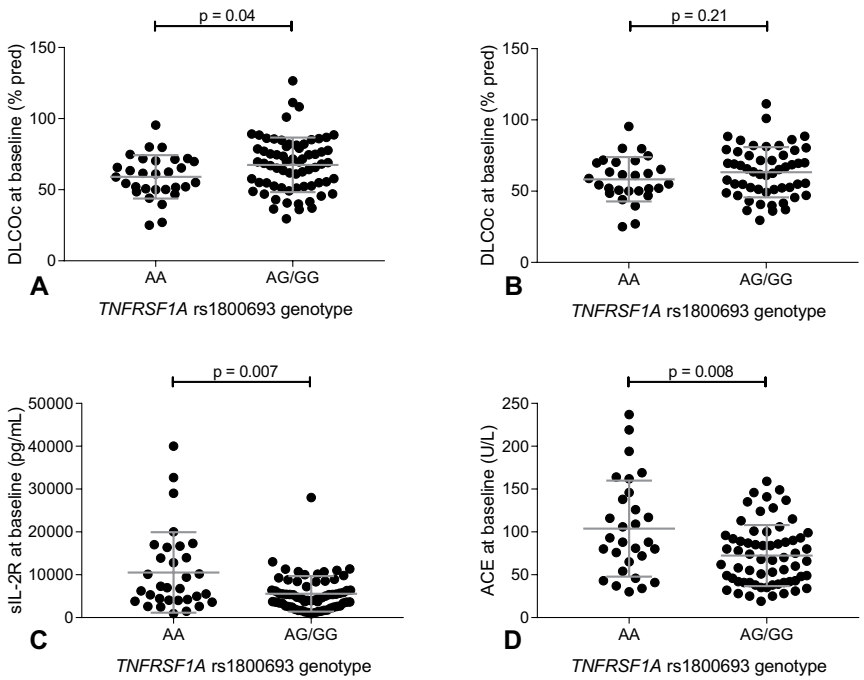


Figure 2. Baseline DLCOc, sIL-2R and ACE according to *TNFRSF1A* rs1800693 genotype.
A) baseline DLCOc in all patients, B) baseline DLCOc in patients with pulmonary involvement, C) baseline sIL-2R in all patients, D) baseline ACE in all patients. DLCOc: diffusing capacity of the lung for carbon monoxide corrected for hemoglobin; % pred: % predicted; sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme. Grey bars represent mean ± SD.

Regarding *TNFRSF1B* T196G, the change in ACE was significantly different between patients with the GG genotype compared to carriers of the T allele (TG+TT): patients with the GG genotype showed a mean increase in ACE of 15 U/L whereas carriers of the T allele showed a mean decrease in ACE of 25 U/L after six months of infliximab treatment (p=0.02, Table 3, Figure 3). Finally, HLA-DRB1*0301 negative patients (tag SNP rs2040410 GG) showed a significantly higher reduction in sIL-2R compared to HLA-DRB1*0301 positive patients (tag SNP rs2040410 GA+AA) (3996 versus 2283 pg/mL, p=0.03, Table 3, Figure 3).

No statistically significant effect of *TNF* G-308A, *FCGR2A* C131T, *FCGR3A* G-158T and HLA-DRB1*1501 tag SNP rs3135388 were found in treatment outcome after six months of infliximab treatment.

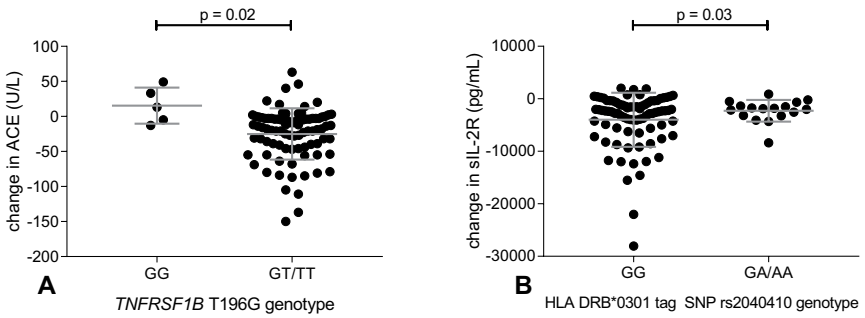


Figure 3. Change in ACE and sIL-2R according to *TNFRSF1B* T196G genotype and HLA-DRB1*0301 tag SNP rs21040410 genotype after six months of infliximab treatment.
A) change in ACE according to *TNFRSF1B* T196G genotype, B) change in sIL-2R according to HLA-DRB*0301 tag SNP rs21040410 genotype, where tag SNP rs2040410A was used to capture HLA-DRB1*0301. sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme. Grey bars represent mean ± SD.

TNF G-308A genotype and response score after one year of treatment

In 71 patients infliximab treatment was continued for one year or more. No association between *TNF* -308 GG genotype and response (improved versus stable/deteriorated) was found (p=0.63, OR 1.31 95% CI 0.44 - 3.95).

6

DISCUSSION

Response to anti-TNF therapy in sarcoidosis shows great variability between patients. The cause of this variability is unknown and it is therefore difficult to predict a patient's response. We studied genetic variations located in several genes that have previously been associated with response to anti-TNF agents in other immune-mediated inflammatory diseases or are hypothesized to influence the response to infliximab because of functional impact. To our knowledge this is the first study to investigate the influence of genetic variations in *TNFRSF1A*, *TNFRSF1B*, *FCGR2A*, *FCGR3A* and *HLA-DRB1* on response to infliximab in severe sarcoidosis.

We found that the *TNFRSF1A* rs1800693 genotype associates with the change in pulmonary function and inflammatory biomarkers after six months of infliximab therapy: patients with the *TNFRSF1A* rs1800693 AA genotype had a better outcome but also a more severe disease activity at baseline compared to carriers of the G allele. The gene *TNFRSF1A* codes for the TNF receptor 1 (TNFR1). This is one of the main receptors that exerts the pro-inflammatory response and apoptosis-related signaling of TNF [15,16]. In rheumatoid arthritis and inflammatory bowel disease several genetic variations in *TNFRSF1A* have been studied as predictors of response to infliximab. But the majority of studies did not detect an association between these genetic variations in *TNFRSF1A* and response to infliximab and the interest in this gene has declined [17-21]. Recently,

a genome-wide association study (GWAS) reported the genetic variant rs1800693 in *TNFRSF1A* to be associated with multiple sclerosis, where presence of the G allele increased the risk for disease [22]. Whether there is an association between this particular genetic variation in *TNFRSF1A* and the response to infliximab has not been studied before in immune-mediated inflammatory diseases. Rs1800693 is located in the sixth intron of the *TNFRSF1A* gene, proximal to exon 6/intron 6 splice site. The effect of this genetic variation on a cellular level has been studied *in vitro* by Gregory *et al.* [23]. In a minigene splicing assay the G allele resulted in skipping of the exon 6. In primary human immune cells the presence of the G allele correlated with increased expression of an isoform of TNFR1 lacking exon 6 (Δ6-TNFR1) [23]. No Δ6-TNFR1 was observed at the surface of transfected cells. Instead, a higher level of Δ6-TNFR1 was detected in TNFR1-transfected HEK293T supernatants. Therefore, in patients carrying the G allele, expression of TNFR1 on the cell surface may be lower and the level of soluble TNFR1 may be higher. Moreover, Gregory *et al.* [23] showed that soluble Δ6-TNFR1 can bind TNF and thereby neutralize the ability of TNF to signal through membrane-bound TNFR1. In our cohort, the patients carrying the G allele, which is the allele that results in the formation of Δ6-TNFR1, was associated with less improvement in DLCOc and a lower decrease in sIL-2R and ACE. We hypothesize that this is caused by a lower expression of membrane-bound TNFR1 in patients carrying the G allele, resulting in less binding places for TNF to signal through. Furthermore, expression of soluble TNFR1 (consisting of TNFR1 and Δ6-TNFR1) that can neutralize TNF may be higher in these patients. Therefore, sarcoidosis may not be as TNF-driven in G allele carrier patients as in patients with the AA genotype and therefore inhibiting TNF with infliximab has less effect. Future studies are needed to explore the potential implications of these findings. In sarcoidosis, no studies on differential expression of membrane-bound TNFR1 or soluble TNFR1 in relation to *TNFRSF1A* rs1800693 genotype have been reported.

In our study, *TNFRSF1B* T196G was found to associate with the change in ACE. In T allele carrier patients the mean decrease in ACE was 25 U/L, while in patients with the GG genotype a mean increase in ACE of 15 U/L was observed. The genetic variation *TNFRSF1B* T196G has previously been associated with response to infliximab in candidate gene studies in Crohn's disease and rheumatoid arthritis, but contradictory results have been reported. Some studies described a better outcome in patients carrying the T allele, similar to what was found in our study [24]. However, other studies found an opposite effect [19] or did not detect an association [18,20,25]. Because the minor allele frequency in *TNFRSF1B* T196G is low, many studies are underpowered. In our study the group with the GG genotype consisted of only five patients. Larger studies are needed to determine the influence of this genetic variation on the treatment with infliximab.

We also studied two tag SNPs of *HLA-DRB1* that have previously been associated with the disease course of sarcoidosis: HLA-DRB1*0301 has been associated with a good prognosis and resolving disease, whereas HLA-DRB1*1501 was overrepresented in the group with persistent disease [26,27]. In our study, patients carrying the HLA-DRB1*0301 tag SNP rs2040410A allele, that has been associated with a resolving form of the disease, showed a smaller decrease in sIL-2R compared to patients not carrying this allele. We did not find an explanation for this finding.

We were not able to replicate the association of *TNF* G-308A with response after one year that was previously published by Wijnen *et al.* [5]. In rheumatoid arthritis and inflammatory bowel disease this genetic variation has been studied extensively with both positive and negative outcome [7,8]. Although we used the same response criteria, we could not replicate the previously published association. A possible explanation for our different result is that in our population the treatment effect was evaluated after six months of treatment and in case of disease progression treatment was discontinued. Therefore, the study population after one year of treatment comprises a differently selected group of patients compared to the patient population evaluated by Wijnen *et al.* [5] where treatment was only evaluated after one year.

To our knowledge, this is the first study to investigate multiple genetics variations in patients with severe sarcoidosis treated with infliximab. Our findings, if confirmed in other studies, may aid in selecting severe sarcoidosis patients who are most likely to respond to infliximab treatment.

CONCLUSION

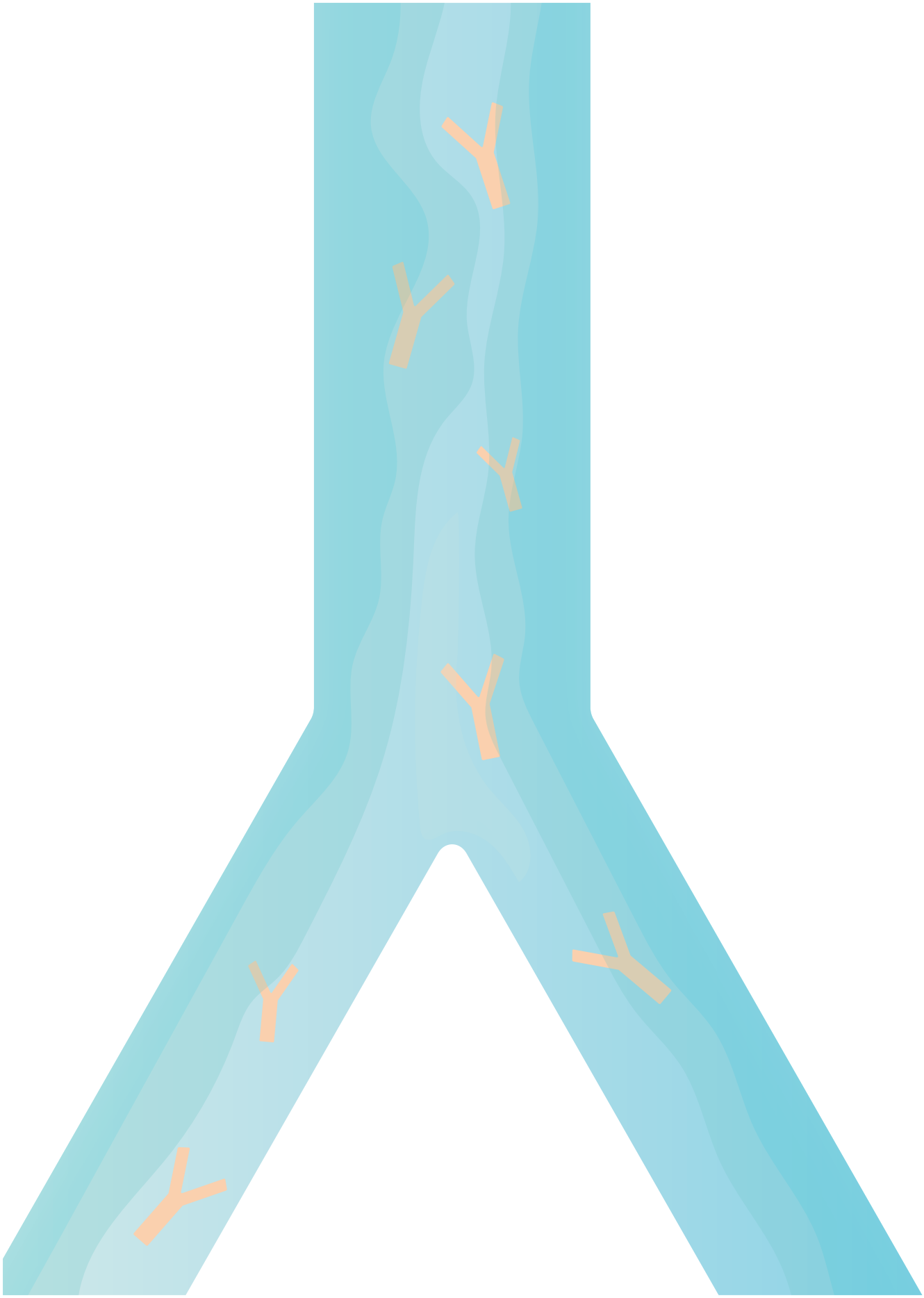
Genetic variants *TNFRSF1A* rs1800693, *TNFRSF1B* T196G and absence of HLA-DRB*0301 associate with changes in clinical and inflammatory parameters in patients with severe sarcoidosis. These findings, if confirmed in other studies, may assist in selecting severe sarcoidosis patients who are most likely to respond to infliximab treatment.

REFERENCES

1. Ziegenhagen MW, Benner UK, Zissel G, Zabel P, Schlaak M, Muller-Quernheim J. Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am J Respir Crit Care Med* 1997; 156: 1586-1592.
2. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
3. Crommelin HA, Vorselaars AD, van Moorsel CH, Korenromp IH, Deneer VH, Grutters JC. Anti-TNF therapeutics for the treatment of sarcoidosis. *Immunotherapy* 2014; 6: 1127-1143.
4. Vorselaars AD, Crommelin HA, Deneer VH, Meek B, Claessen AM, Keijsers RG, van Moorsel CH, Grutters JC. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J* 2015; 46: 175-185.
5. Wijnen PA, Cremers JP, Nelemans PJ, Erckens RJ, Hoitsma E, Jansen TL, Bekers O, Drent M. Association of the TNF-alpha G-308A polymorphism with TNF-inhibitor response in sarcoidosis. *Eur Respir J* 2014; 43: 1730-1739..
6. Prieto-Perez R, Cabaleiro T, Dauden E, Abad-Santos F. Gene polymorphisms that can predict response to anti-TNF therapy in patients with psoriasis and related autoimmune diseases. *Pharmacogenomics J* 2013; 13: 297-305.
7. Prieto-Perez R, Almoguera B, Cabaleiro T, Hakonarson H, Abad-Santos F. Association between Genetic Polymorphisms and Response to Anti-TNFs in Patients with Inflammatory Bowel Disease. *Int J Mol Sci* 2016; 17: 225.
8. Lee YH, Ji JD, Bae SC, Song GG. Associations between tumor necrosis factor-alpha (TNF-alpha) -308 and -238 G/A polymorphisms and shared epitope status and responsiveness to TNF-alpha blockers in rheumatoid arthritis: a metaanalysis update. *J Rheumatol* 2010; 37: 740-746.
9. Umicevic Mirkov M, Cui J, Vermeulen SH, Stahl EA, Toonen EJ, Makkinje RR, Lee AT, Huizinga TW, Allaart R, Barton A, Mariette X, Miceli CR, Criswell LA, Tak PP, de Vries N, Saevarsdottir S, Padyukov L, Bridges SL, van Schaardenburg DJ, Jansen TL, Dutmer EA, van de Laar MA, Barrera P, Radstake TR, van Riel PL, Scheffer H, Franke B, Brunner HG, Plenge RM, Gregersen PK, Guchelaar HJ, Coenen MJ. Genome-wide association analysis of anti-TNF drug response in patients with rheumatoid arthritis. *Ann Rheum Dis* 2013; 72: 1375-1381.
10. Xie X, Zhang D, Chen JW, Tian J, Ling GH, Li F. Pharmacogenomics of biological treatment in rheumatoid arthritis. *Expert Opin Biol Ther* 2014; 14: 157-164.
11. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
12. Vercoulen J, Alberts M, Bleijenberg G. [De Checklist Individual Strength (CIS)]. *Gedragstherapie* 1999: 131-136.
13. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap) - a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42: 377-381.
14. de Bakker PIW, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, Ke X, Monsuur AJ, Whittaker P, Delgado M, Morrison J, Richardson A, Walsh EC, Gao X, Galver L, Hart J, Hafler DA, Pericak-Vance M, Todd JA, Daly MJ, Trowsdale J, Wijmenga C, Vyse TJ, Beck S, Murray SS, Carrington M, Gregory S, Deloukas P, Rioux JD. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 2006; 38: 1166-1172.
15. Szondy Z, Pallai A. Transmembrane TNF-alpha reverse signaling leading to TGF-beta production is selectively activated by TNF targeting molecules: Therapeutic implications. *Pharmacol Res* 2017; 115: 124-132.
16. Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008; 117: 244-279.
17. Medrano LM, Taxonera C, Marquez A, Barreiro-de Acosta M, Gomez-Garcia M, Gonzalez-Artacho C, Perez-Calle JL, Bermejo F, Lopez-Sanroman A, Martin Arranz MD, Gisbert JP, Mendoza JL, Martin J, Urcelay E, Nunez C. Role of TNFRSF1B polymorphisms in the response of Crohn's disease patients to infliximab. *Hum Immunol* 2014; 75: 71-75.
18. Mascheretti S, Hampe J, Kuhbacher T, Herfarth H, Krawczak M, Folsch UR, Schreiber S. Pharmacogenetic investigation of

the TNF/TNF-receptor system in patients with chronic active Crohn's disease treated with infliximab. *Pharmacogenomics J* 2002; 2: 127-136.

19. Steenholdt C, Enevold C, Ainsworth MA, Brynskov J, Thomsen OO, Bendtzen K. Genetic polymorphisms of tumour necrosis factor receptor superfamily 1b and fas ligand are associated with clinical efficacy and/or acute severe infusion reactions to infliximab in Crohn's disease. *Aliment Pharmacol Ther* 2012; 36: 650-659.
20. Matsukura H, Ikeda S, Yoshimura N, Takazoe M, Muramatsu M. Genetic polymorphisms of tumour necrosis factor receptor superfamily 1A and 1B affect responses to infliximab in Japanese patients with Crohn's disease. *Aliment Pharmacol Ther* 2008; 27: 765-770.
21. Pierik M, Vermeire S, Steen KV, Joossens S, Claessens G, Vlietinck R, Rutgeerts P. Tumour necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatory bowel disease and their association with response to infliximab. *Aliment Pharmacol Ther* 2004; 20: 303-310.
22. De Jager PL, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT, Piccio L, Raychaudhuri S, Tran D, Aubin C, Briskin R, Romano S, International MS Genetics Consortium, Baranzini SE, McCauley JL, Pericak-Vance MA, Haines JL, Gibson RA, Naeglin Y, Uitdehaag B, Matthews PM, Kappos L, Polman C, McArdle WL, Strachan DP, Evans D, Cross AH, Daly MJ, Compston A, Sawcer SJ, Weiner HL, Hauser SL, Hafler DA, Oksenberg JR. Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat Genet* 2009; 41: 776-782.
23. Gregory AP, Dendrou CA, Attfield KE, Haghikia A, Xifara DK, Butter F, Poschmann G, Kaur G, Lambert L, Leach OA, Promel S, Punwani D, Felce JH, Davis SJ, Gold R, Nielsen FC, Siegel RM, Mann M, Bell JI, McVean G, Fugger L. TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* 2012; 488: 508-511.
24. Rooryck C, Barnetche T, Richez C, Laleye A, Arveiler B, Schaeverbeke T. Influence of FCGR3A-V212F and TNFRSF1B-M196R genotypes in patients with rheumatoid arthritis treated with infliximab therapy. *Clin Exp Rheumatol* 2008; 26: 340-342.
25. Vasilopoulos Y, Manolika M, Zafiriou E, Sarafidou T, Bagiatis V, Kruger-Krasagaki S, Tosca A, Patsatsi A, Sotiriadis D, Mamuris Z, Roussaki-Schulze A. Pharmacogenetic analysis of TNF, TNFRSF1A, and TNFRSF1B gene polymorphisms and prediction of response to anti-TNF therapy in psoriasis patients in the Greek population. *Mol Diagn Ther* 2012; 16: 29-34.
26. Grunewald J, Eklund A, Olerup O. Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients. *Am J Respir Crit Care Med* 2004; 169: 696-702.
27. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J. HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1997; 156: 1601-1605.



7

Pharmacokinetics and exposure-response relationship of infliximab in severe sarcoidosis

Heleen A Crommelin
Adriane DM Vorselaars
Coline HM van Moorsel
Johannes H Proost
Jan C Grutters
Vera HM Deneer

Submitted

ABSTRACT

Aims: Sarcoidosis is a multi-organ disease characterized by inflammation and non-caseating granulomas. In severe sarcoidosis, infliximab is an effective off-label third-line therapeutic. The aim of this study was to describe the pharmacokinetics and exposure-response relationship of infliximab in severe sarcoidosis.

Methods: Sarcoidosis patients treated with infliximab (n=68) 5 mg/kg at week 0, 2 and every four weeks thereafter, were studied for two years. Serum samples were collected at every dose just before and one hour after the end of infusion during the first six months and thereafter every three months. Response on clinical and inflammatory parameters and a composite response score were determined after six months of treatment. A pharmacokinetic model was developed using NONMEM.

Results: Population pharmacokinetic estimates (typical value (relative standard error)) in the final covariate model were clearance (CL) 0.276 L/day (3.2%), volume of central compartment (V1) 3.16 L (1.6%), intercompartmental clearance (Q) 0.177 L/day (21%) and volume of peripheral compartment (V2) 1.49 L (11%). Interindividual variability for CL, V1 and V2 were 23.5%, 10.9% and 75.4%, respectively. Covariate analyses showed that V1 increased with baseline body surface area and CL increased with positive antibodies toward infliximab (ATI) status, low baseline serum albumin and high body weight. No association with inflammatory activity or genotype and no exposure-response relationship were found.

Conclusions: Baseline body surface area, body weight, serum albumin and ATI status were found to influence the pharmacokinetics of infliximab in severe sarcoidosis. No exposure-response relationship was found, indicating overdosing in the current treatment protocol.

INTRODUCTION

Sarcoidosis is a multi-organ disease characterized by inflammation and non-caseating granulomas. Virtually any organ can be affected but in most patients (> 95%) the lungs are involved [1]. The etiology of sarcoidosis remains unclear, but studies have shown an increased secretion of tumor necrosis factor (TNF) by alveolar macrophages [2]. Systemic treatment is indicated when the disease is threatening organ function or severely deteriorates quality of life. First-line treatment consists of corticosteroids, and second-line treatment consists of cytotoxic and immunomodulatory agents such as methotrexate (MTX) or azathioprine (AZA). However, some patients are refractory to these agents or may suffer from serious side effects [3,4]. In these patients infliximab is an effective, off-label third-line therapeutic according to international guidelines and one of the final treatment options in these severely ill patients [5,6]. Infliximab is a recombinant chimeric IgG monoclonal antibody specifically targeting soluble and membrane-bound TNF. Infliximab is approved for the use in inflammatory diseases such as rheumatoid arthritis, Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriatic arthritis and psoriasis [7]. Although the mechanism of action of infliximab across these different inflammatory diseases is similar, the optimal treatment protocol with infliximab varies by disease. This is illustrated by the difference in approved dosing regimens: in rheumatoid arthritis the approved maintenance dose is 3 mg/kg every eight weeks, whereas in ankylosing spondylitis, 5 mg/kg every six weeks is approved and in inflammatory bowel diseases 5 mg/kg every eight weeks [7]. In sarcoidosis the recommended maintenance dosing regimen is 5 mg/kg every four weeks. This intensive dosing regimen in sarcoidosis is primarily based on expert opinion [5].

Across different diseases differential drug disposition of infliximab has been associated with several factors: body weight has been associated with both volume of distribution and clearance. Body weight differs across different patient populations treated with infliximab: median body weight in sarcoidosis patients is greater than in populations with ankylosing spondylitis, rheumatoid arthritis and inflammatory bowel diseases [8-17]. Moreover, pre-treatment CRP has been associated with infliximab concentration and exposure in rheumatoid arthritis and ulcerative colitis, and pre-infusion CRP with infliximab elimination in rheumatoid arthritis and Crohn's disease, most likely because CRP is regarded as a surrogate for TNF burden [8-12]. But, in sarcoidosis inflammatory activity is best described by serum soluble interleukin-2 receptor (sIL-2R), angiotensin-converting enzyme (ACE) and standardized maximum uptake value (SUVmax) on ^{18}F-fluorodexoyglucose positron emission tomography (^{18}F-FDG PET) scan rather than by CRP [18-22].

Clearly, sarcoidosis patients treated with infliximab differ from other infliximab treated populations, and factors affecting the pharmacokinetics and effect of infliximab may also differ. But the pharmacokinetics of infliximab and the relation with effect have never been studied in sarcoidosis. Knowledge on factors that influence the pharmacokinetics of infliximab can help to optimize and rationalize the infliximab dosing regimen in severe sarcoidosis.

Therefore, the aim of this study was to describe the pharmacokinetics and exposure-response relationship of infliximab in severe sarcoidosis, and to assess the influence of parameters known to influence infliximab pharmacokinetics in other inflammatory diseases tailored to sarcoidosis.

METHODS

Ethics

This study was approved by the institutional medical research ethics committee of St Antonius Hospital Nieuwegein and was carried out in concordance with ICH Guidelines for Good Clinical Practice. Written informed consent was obtained from all individual participants in the study. The study was registered at www.trialregister.nl with identifier number NTR3895.

Study population

This prospective, single-center study of infliximab-treated sarcoidosis patients was conducted between January 2011 and September 2014 at the Interstitial Lung Diseases Centre of Excellence, Department of Pulmonology at St Antonius Hospital Nieuwegein. The design of the study has been reported previously [6]. Briefly, all sarcoidosis patients who were treated with infliximab (Remicade®) therapy at St Antonius Hospital (Nieuwegein, The Netherlands) between January 2011 and April 2013 were invited to participate in this prospective, open-label, cohort study. Both patients starting infliximab treatment and patients who were already receiving infliximab treatment were eligible. All patients had an established diagnosis of sarcoidosis, according to American Thoracic Society/European Respiratory Society criteria [1] and were unresponsive to corticosteroids and second-line treatment, most often MTX or AZA, or experienced severe side effects from these agents (*e.g.* worsening diabetes, psychological deterioration or liver function disorders).

Treatment administration and blood sampling

Patients received 5 mg/kg bodyweight of infliximab as a two hour intravenous infusion at weeks 0 and 2 and every four weeks thereafter. The interval between infusions was fixed at four weeks during the first six months of treatment, unless patients suffered from infection, resulting in postponing infliximab administration. Infusion duration and interval between infusions after six months could be prolonged according to judgement of the treating physician (*e.g.* infusion duration could be lengthened because of suspicion of allergic reaction or the interval could be prolonged because of an infection or because of treatment tapering).

Blood samples were collected to measure infliximab serum concentration. Infliximab concentrations were determined both in predose serum samples and in samples collected one hour after the end of the infusion. During the first six months of treatment blood samples were drawn at every dose, thereafter every three months. Additionally, during the first six months patients were

asked twice to provide a serum sample two weeks after infliximab administration. In patients who were clinically suspected of having developed antibodies toward infliximab (ATIs) (*e.g.* allergic reaction, loss of response), ATIs were determined using radio-immunoassay. Patient follow-up lasted up to two years.

Analytical methods

Infliximab serum concentrations were measured using a validated enzyme-linked immunosorbent assay (ELISA) (Sanquin, The Netherlands) [10]. The lower limit of quantification was 0.48 µg/mL at a 1:500 dilution. The intra-assay precision, expressed as a coefficient of variation, ranged from 10.1% to 16.9%, whereas the interassay precision ranged from 11.3% to 19.4%. In samples collected one hour after the end of the infusion a dilution of 1:10,000 was used. Sera exceeding 200 µg/mL were further diluted.

ATIs were determined in predose samples by Sanquin Diagnostics, Amsterdam, The Netherlands, using radioimmunoassay [23]. Patients were classified as ATI positive when ATIs were detected in any sample analyzed for ATIs.

A total of 27 erroneous data points were excluded from the analysis. Data were deemed erroneous if the trough concentration was greater than the one hour post-dose concentration, when time of infusion end preceded time of infusion start or when sampling time post infusion preceded time of infusion stop (most probably due to switch between pre- and post dose blood collection sample tubes or a recording error).

Covariate data

Patient files were reviewed by investigators and predefined relevant covariates to be investigated were recorded. Data were treated as continuous, categorical or binominal data. The baseline covariate value was defined as the last recorded value prior to initiation of infliximab treatment. If a baseline covariate value was missing, the median value was imputed in case of a continuous covariate and for a categorical or binominal covariate, the value with the highest proportion of patients was imputed. Demographic covariates were age, sex, race (i.e. Caucasian, black, other), body weight (BW), body mass index (BMI), lean body weight (LBW) and body surface area (BSA). LBW was calculated as described by Janmahasatian *et al.* [24] and BSA was calculated using the Dubois formula. Inflammatory biomarkers were sIL-2R, ACE, CRP and SUVmax on ^{18}F-FDG PET scan of the lung, the hilar and mediastinal region and, if applicable, the index organ other than the lungs. Additional covariates were serum albumin, use of low dose concomitant immunomodulators (MTX, AZA, leflunomide and/or corticosteroids) during the study period and ATI status. Additionally, patients were genotyped for *TNF* G-308A (rs1800629) and *FCGR3A* V-158F (rs396991). Genomic deoxyribonucleic acid was extracted from peripheral blood of each individual using a standard method. Pre-designed taqman single nucleotide polymorphism genotyping assays and an ABI 7500Fast analyzer (Applied Biosystems, Foster City, CA) were used to genotype *TNF* G-308A (rs1800629) and *FCGR3A* V-158F (rs396991). Genotypes were tested as categorical and binom-

inal (*e.g.* carrier ship) covariates. An interoccasional variability term was also included to assess any potential variability in the pharmacokinetic properties of infliximab between the induction period (represented by infliximab concentration data in weeks 0 to 6) and the maintenance period (after week 6 to study end).

ACE levels were not taken into account in patients who were using an ACE inhibitor. The same applied for sIL-2R levels in patients with kidney dysfunction (eGFR < 60 mL/min) [25]. SUVmax values were only available in patient who started infliximab during the study period, not in patients who were already on infliximab when the study started.

Pharmacokinetic analysis

Software

Infliximab concentration-time data were analyzed using NONMEM (version 7.3; ICON Development Solutions, Ellicott City, MD, USA) with Pirana, PsN and R/Xpose as modelling environment [26].

Data below the quantification limit

Only 2.4% (28/1181) of infliximab concentrations were below the quantification limit (BQL) (0.48 µg/mL). BQL concentrations were replaced by 0.5 BQL (0.24 µg/mL). Data were screened for consecutive BQL levels within one dosing interval but none were observed.

Data records

In all patients previous infliximab treatment was recorded. In patients previously treated with infliximab at St Antonius Hospital Nieuwegein dosing records of infliximab as of April 2009 were included in the pharmacokinetic analysis.

After six months of treatment blood samples were drawn only every three months and therefore time of start and stop of infusion was missing in the infusions in between. Time of infusion start for these infusions was set at 11:00 am and infusion rate was adopted based on previous infusion rates for that particular patient.

Population pharmacokinetic model development

Pharmacokinetic data were described using the first-order conditional estimation method with interaction (FOCE-I) in NONMEM. One-, two- and three-compartment models with zero-order infusion and first-order distribution and elimination were tested during structural model development. Interindividual variability (IIV) of the pharmacokinetic parameters was explored by stepwise addition of IIV to parameters. Individual values of the pharmacokinetic parameters were described using exponential models:

$$P_i = \theta * \exp(\eta_i)$$

where Pi is the individual subject parameter in the ith patient, θ is the estimate of the population mean or typical value of the parameter and ηi is the individual specific random effect for the

ith patient, which is drawn from a distribution with a mean of zero and variance of ω^2. Residual variability was estimated by proportional, additive or combined error models. The combined error model was tested assuming two different sources of error and assuming one source of residual error [27].

Only a model that converged with a successful covariance step was considered for further analysis. Goodness of fit for a model was assessed using plots of observed infliximab concentrations (DV) versus population-predicted (PRED) and individual-predicted concentrations (IPRED) (i.e. DV vs PRED and DV vs IPRED, respectively). The residual error components of models were assessed via scatter plots of conditional weighted residuals (CWRES) versus PRED and CWRES versus time after dose. Smooth nonparametric trend curves (locally weighted scatter plot smoother (LOESS)) were included in the graphs as appropriate. The best model was determined by visual comparison of goodness of fit and by comparing objective function value (OFV).

Covariate analysis

Potential covariates were assessed using linear, piece-wise linear, exponential and power equations for continuous covariates and a linear equation for categorical covariates on systemic clearance (CL) and volume of the central compartment (V1). First, covariates were added using a stepwise forward-inclusion procedure with a p-value of less than 0.05, which corresponds to a difference in OFV > 3.84. Then, a stepwise backward-deletion procedure was used with a stricter criterion of $p < 0.005$: only covariates associated with an increase in OFV of at least 7.88 were retained in the model. This stricter criterion was used because of multiple comparisons that were performed during the forward inclusion procedure [28].

The effect of covariates was assessed on all parameters, but because covariates on intercompartmental clearance (Q) and volume of the peripheral compartment (V2) frequently resulted in an unsuccessful covariance step, ultimately covariates were assessed only on CL and V1.

Population pharmacokinetic model evaluation

The internal validity of the population pharmacokinetics and models was assessed by the bootstrap re-sampling method using 2000 replicates. Non-parametric 95% confidence intervals (95% CIs) were obtained from the bootstrap replicates. Parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original dataset.

Pharmacodynamics

Only patients who started infliximab treatment at the time of study start were included in this pharmacodynamic analysis. The area under the infliximab concentration-time curve (AUC) from treatment initiation to six months of treatment, which was when treatment response was evaluated, was computed using NONMEM for each patient. Clinical and inflammatory parameters and a composite response score were evaluated at baseline and after six months in patients. Clinical parameters were pulmonary function tests, i.e. forced vital capacity (FVC) and diffusing capacity

of the lung for carbon monoxide corrected for hemoglobin (DLCOc). Inflammatory parameters were ACE, sIL-2R, CRP and SUVmax on ^{18}F-FDG PET scan of the pulmonary parenchyma and, if applicable, the index organ other than the lungs. The composite clinical response score, which included organ function, inflammatory biomarkers and quality of life, was previously described [6]. Visual examination of scatterplots, and univariate and multivariate linear regression were used to test whether AUC correlated with pharmacodynamic parameters, except for the response score, which was tested using ordinal regression. Linear and ordinal regression were performed using SPSS for Windows (version 24; IBM, Armonk, NY, USA).

RESULTS

Patients

Data from 68 evaluable patients was used for the pharmacokinetic analysis. Most patients (n=55, 81%) were included at the time of infliximab initiation; the other 13 patients were already receiving infliximab at the time of study start. Median body weight was 84 kg (range 56 to 140) (Table 1). Median follow-up time was 14.7 months (range 0.5 - 26.4). A total of 1,181 serum samples were available for the pharmacokinetic analysis. Median infliximab trough level was 17.9 µg/mL (n=589) and median top level was 150.5 µg/mL (n=511). Median level two weeks after infliximab infusion was 41.8 µg/mL (n=81). ATIs were detected in pre-infusion serum samples of six patients, 18 patients were tested negative and in 44 patients ATIs were not measured because there was no clinical suspicion for presence of ATIs.

Structural pharmacokinetic model

The pharmacokinetics of infliximab was best described by a two-compartment model with a combined proportional and additive error model assuming one source of variation. Stepwise addition of IIV on parameters CL, V1 and V2 resulted in a significant improvement in model fit and OFV. The pharmacokinetic parameters are summarized in Table 2. The η-shrinkage for CL was 2.1%, for V1 8.1% and for V2 21% and ε-shrinkage was 7.0%.

Table 1. Summary of patient characteristics of the study subjects at baseline.

Patient characteristics	
Subjects	68
Demographic characteristics at study start	
Age at study start (years)	48.4 [29.1-67.6]
Male sex	44 (63)
Body weight (kg)	84 [56-140]
Body mass index (kg/m^2)	27.3 [18.9-44.2]
Lean body weight (kg)	60.9 [37.0-83.4]
Body surface area (m^2)	2.01 [1.57-2.54]
Ethnicity	
Caucasian	60 (88)
Black	6 (9)
Other	2 (3)
New or ongoing	
New	55 (81)
Ongoing	13 (19)
Disease duration at study start (years)	4.3 [0.7-32.6]
Medication prior to infliximab	
Corticosteroids	66 (97)
Methotrexate	58 (85)
Azathioprine	7 (10)
Leflunomide	1 (1)
Plaquenil	9 (13)
Infliximab	15 (22)
Adalimumab	2 (3)
None	0 (0)
Use of ≥ 2 different drugs prior to infliximab	59 (87)
Concomitant immunosuppressive medication	
Corticosteroids	22 (32)
Methotrexate	56 (82)
Azathioprine	3 (4)
Leflunomide	1 (1)
None	0 (0)
Inflammatory biomarkers	
sIL-2R (pg/mL)	5366 [899-40200]†
ACE (U/L)	82 [6-237]‡
SUVmax total (incl index localization)	8.2 [1.7-22.1]
SUVmax pulmonary parenchyma	5.6 [0.6-19.3]
SUVmax mediastinum	5.5 [1.7-16.1]
CRP (mg/L)	4.5 [0.5-43]
Albumin (g/L)	43.6 [26.3-50.1]
Genotype frequencies, n	
TNF G-308A (rs1800629) GG/AG/AA	48/16/3§
FCGR3A V-158F (rs396991) AA/CA/CC	37/24/6§

Data are expressed as median [range] or n (%), unless stated otherwise. sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme; SUVmax: maximum standardized uptake value on ^{18}F-fluorodexoyglucose positron emission tomography (^{18}F-FDG PET) scan.

† n=62; ‡ n=58; § for one patient no DNA sample was available.

Table 2. Parameter estimates for the infliximab population pharmacokinetic model.

Parameter	Base model		Final model		Bootstrap*	
	Estimate	%RSE	Estimate	%RSE	Median	95% CI
CL (L/day)	0.304	5.1	0.276	3.2	0.278	0.257, 0.324
V1 (L)	3.18	2.4	3.16	1.6	3.15	2.89, 3.26
Q (L/day)	0.189	43	0.177	21	0.194	0.101, 2.90
V2 (L)	1.50	14	1.49	11	1.55	1.20, 2.87
IIV						
IIV on CL (%)	33.6	26	23.5	19	22.3	18.2, 27.2
IIV on V1 (%)	18.3	21	10.9	22	10.8	8.2, 13.0
IIV on Q (%)	-	-	-	-	-	-
IIV on V2 (%)	76.9	47	75.4	48	72.7	24.1, 113
Residual variability						
Proportional (%)	14.6	5.2	14.7	4.6	14.7	13.2, 16.5
Additional (µg/mL)	0.936	25	0.936	23	0.902	0.398, 1.41
Covariates						
ATI on CL	-	-	0.814	38	0.825	0.180, 2.20
BW on CL	-	-	0.00661	27	0.00654	0.00315, 0.0104
ALB on CL	-	-	-0.975	27	-0.968	-1.67, -0.454
BSA on V1	-	-	0.663	10	0.669	0.514, 0.826

*Calculated from 2000 bootstrap data sets; convergence rate 91.5%
RSE: relative standard error; CL: systemic clearance; V1: volume of central compartment; Q: intercompartmental clearance; V2: volume of peripheral compartment; IIV: interindividual variability; ATI: antibodies toward infliximab status; BW: baseline body weight (in kg); ALB: baseline serum albumin concentration (in g/L); BSA: baseline body surface area (in m^2).

Covariate model

Covariates that were included in the stepwise addition procedure were ATI status, baseline BW, baseline serum albumin and baseline sIL-2R on clearance and baseline BSA on central volume. After backward elimination, the covariates included in the final model were baseline serum albumin, ATI status and baseline BW on clearance, and baseline BSA on central volume. Some of the IIV of the population parameters was explained by inclusion of the covariates, as indicated by a reduction from 33.6% and 18.3% in the base model to 23.5% and 10.9% in the final model for CL and V1, respectively. The final model was:

$$CL = 0.276 * (1 + 0.814 * ATI) * e^{0.00661*(BW-84)} * \left(\frac{ALB}{43.6}\right)^{-0.975}$$

$$V1 = 3.16 * \left(1 + 0.663 * (BSA - 2.00)\right)$$

$$Q = 0.177$$

$$V2 = 1.49$$

where ATI status = 1 for patients who tested positive and 0 for patients who tested negative or who were not clinically suspected for presence of ATIs, BW is body weight at baseline, ALB is serum albumin at baseline and BSA is BSA at baseline.

Goodness of fit plots show that the final model adequately described the observed values (Figure 1). Observed values > 200 µg/mL were slightly underpredicted by the model. No systematic bias was found in CWRES and the mean ± SD of the CWRES was close to zero -0.03 (±0.99), approximating a normal distribution. The terminal half-life for infliximab was about 14 days and the residual error plots showed no systematic deviation over time (Figure 1).

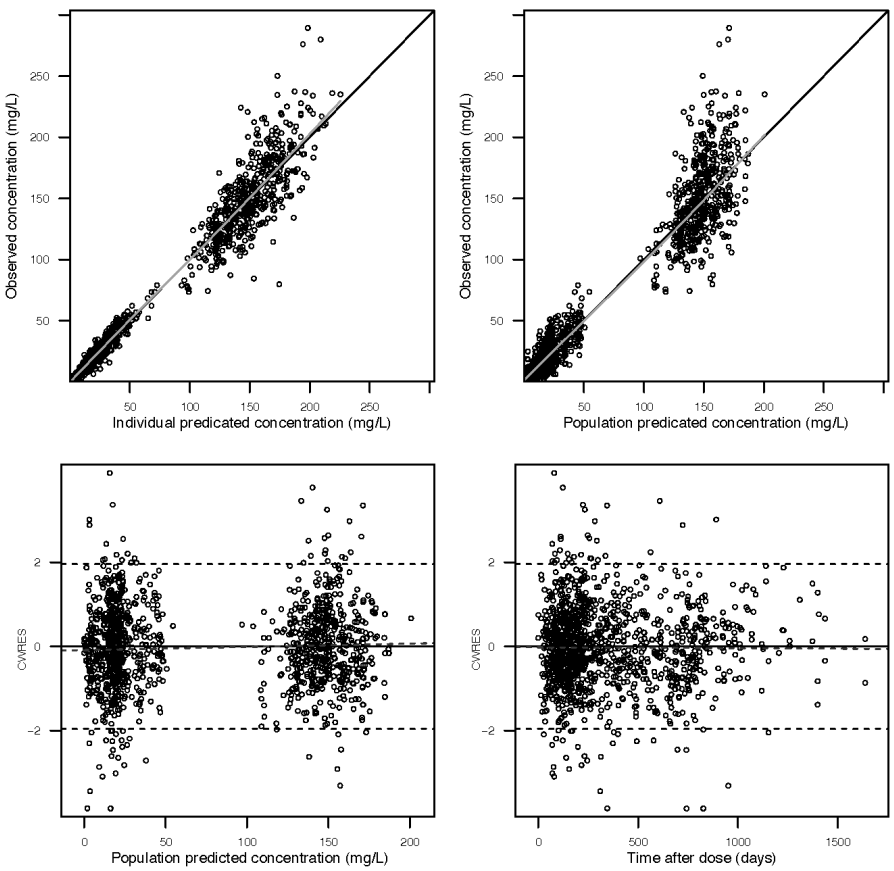


Figure 1. Goodness of fit plots for the final model.
Dashed grey lines represent the locally weighted smoothing of the data. CWRES: conditional weighted residuals, time in days after dose.

The condition number of the final model was 19.8, suggesting that the model was not ill-conditioned as a value greater than 1000 indicates an ill-conditioned model.

Parameter estimates for the final model, including 95% CIs obtained by a bootstrap resampling technique are summarized in Table 2. A great interindividual variation in V2 of 75.4% was observed with a large relative standard error of 48%. However, when IIV on V2 was removed from the model, the OFV increased by 50 points and therefore the IIV on V2 was retained in the model. One patient had an exceptionally high estimated V2 of 26.1 L, compared to the estimated population mean of 1.49 L. This patient initially had normal trough infliximab serum levels compared to other patients, but trough levels decreased to undetectable levels. ATIs were assessed and detected. Treatment was continued and trough levels increased back to initial levels. The patient was again tested for ATIs and test results were negative.

The median values obtained by the bootstrap resampling technique were in good agreement with the point estimates. The significance of the included covariates was further supported by the bootstrap analysis as none of the 95% CIs for the covariate effects included 0.

Effect of BSA and BW

Baseline BSA was a significant covariate of V1, described by a linear relationship, and was the most significant covariate. Baseline BW was a significant covariate of CL. This relationship was best described using an exponential functional form. Figure 2A, panel I displays a scatterplot of the empirical Bayesian estimates (EBEs) of V1 versus BSA; panel II displays a scatterplot comparing the random effect (ETA) of V1 for the base model and panel III for the final model, illustrating the reduction in IIV from the base to final model as a result of the inclusion op BSA as a covariate. Figure 2B provides plots for the relationship between BW and CL.

Effect of serum albumin concentration

A lower baseline serum albumin concentration was associated with a higher infliximab CL (Figure 2C panel I). This relationship was best described by a centered covariate with a power factor. Figure 2C, panels II and III display the association between the random effect of CL (ETA1) with baseline serum for the base model and the final model, respectively.

Effect of antibodies toward infliximab status

A total of six patients were tested positive for ATI during the study period. Mean CL was 81% higher in these patients compared with those who were non-positive or not tested. Figure 2D panel I displays a box plot of CL versus ATI status in the base model. The relationship between ETA1 and ATI status for the base model and final model is depicted in Figure 2D, panel II and III, respectively. The addition of ATI status as a covariate appears to have corrected the difference in clearance between the two groups in the final model.

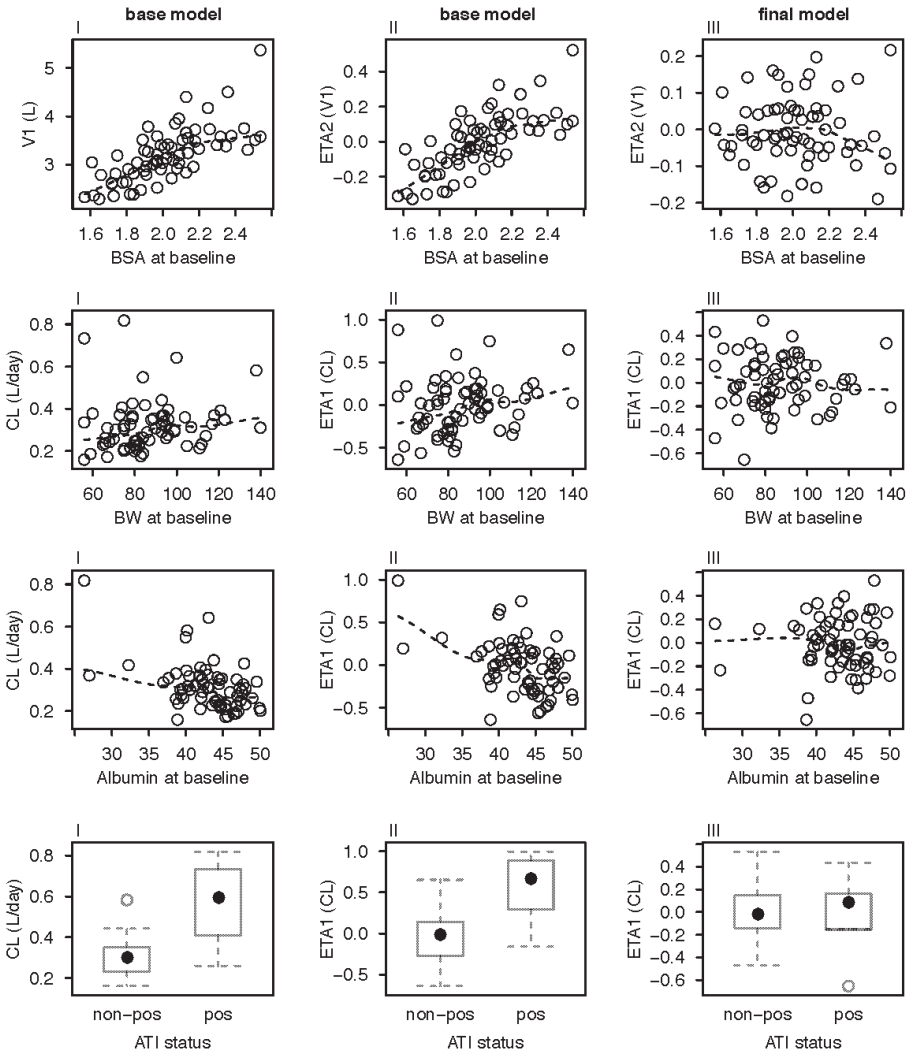


Figure 2. Empirical Bayesian Estimates (EBEs) of parameters (panels I) and ETAs (panels II) in the base model and ETAs (panels III) in the final model.
(A) V1 and ETA2(V1) versus baseline body surface area (BSA), (B) CL and ETA1(CL) versus baseline body weight (BW), (C) CL and ETA1(CL) versus baseline serum albumin, (D) CL, and ETA1(CL) versus antibody toward infliximab (ATI) status. V1: volume of central compartment; CL: systemic clearance; ETA: random effect; non-pos: non-positive; pos: positive. BSA in m^2, BW in kg, serum albumin in g/L.

Pharmacodynamics

In 47 patients clinical and inflammatory parameters were evaluated after six months of treatment (Table 3). A weak but statistically significant correlation between AUC and change in FVC was observed (scatter plots, p=0.035, R=0.311). A high AUC was associated with a low response on FVC (Figure 3). When SUVmax of the pulmonary parenchyma at baseline was added as an independent variable in a multivariate regression analysis, no significant association between AUC and change in FVC was found (p=0.133). No significant correlation between AUC and change in DLCOc, change in inflammatory parameters or response score was found.

Table 3. Patient characteristics in 47 patients in pharmacodynamic analysis before and after the first six months of treatment.

Patient characteristic	
Pulmonary function	
Change in FVC (% pred)	5.6 [-17 to 19]
Change in DLCOc (% pred)	3.5 [-9.2 to 14]
Inflammatory parameters	
Change in sIL-2R (pg/mL)	-3264 [-15530 to 5423]
Change in ACE (U/L)	-21 [-150 to 63]
Change in CRP (mg/L)	-2 [-18 to16]
Change in SUVmax (incl index organ)	-4.7 [-20 to 3.3]
Response score	
0	3 (6)
1	8 (17)
2	18 (38)
3	18 (38)

Data are presented as median [range] or n (%). FVC: forced vital capacity; % pred: % predicted; DLCOc: diffusing capacity of the lung for carbon monoxide corrected for hemoglobin, sIL-2R: soluble interleukin-2 receptor; ACE: angiotensin-converting enzyme; SUVmax: maximum standardized uptake value on ^{18}F-fluorodexoyglucose positron emission tomography (^{18}F-FDG PET) scan. Response score is a composite clinical score which includes organ function, inflammatory biomarkers and quality of life, as previously described [59].

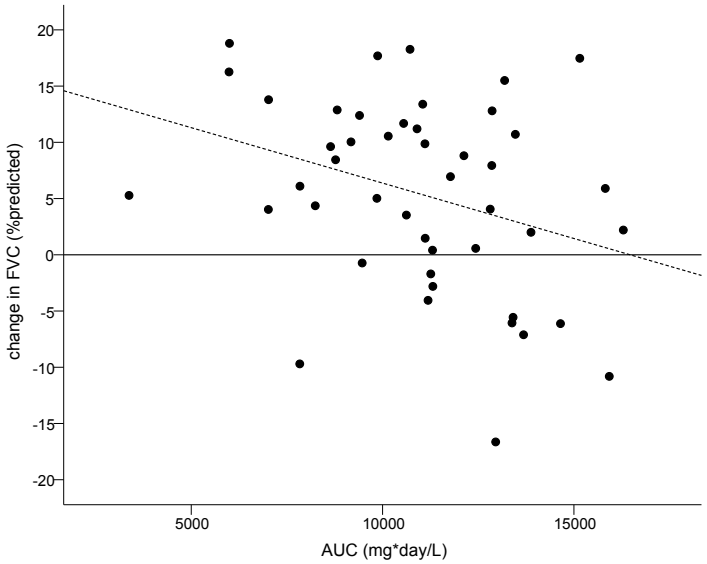


Figure 3. Infliximab area-under-the-curve (AUC) during the first six months of treatment and change in forced vital capacity (FVC) after six months of infliximab treatment.
Correlation between high AUC and low response on FVC (p=0.035, R=0.311).

DISCUSSION

This is the first study on the pharmacokinetics and pharmacodynamics of infliximab in severe sarcoidosis patients and uses long-term clinical data. In our study infliximab concentrations were satisfactorily described by a two-compartment model. The factors baseline BW, ATI status and baseline serum albumin concentration were found to have a significant effect on infliximab clearance. The factor baseline BSA has a significant effect on central volume. IIV on intercompartmental clearance was not included in the final model because shrinkage was observed. This has been described before in infliximab pharmacokinetic modelling [11,16]. The final population pharmacokinetic model described the observed concentration-time profiles of infliximab reasonably well. The goodness of fit plots for the final model showed a slight bias toward underprediction for a low percentage of observed serum infliximab concentrations with values > 200 µg/mL. We could not identify an exact cause for this. Nevertheless, bootstrap analysis showed that the final model adequately and accurately estimated the pharmacokinetic parameters and had reasonable predictability.

The present study found that serum albumin was an influential covariate of clearance in sarcoidosis. High baseline serum albumin levels were associated with low infliximab clearance, and low baseline serum albumin levels were associated with high infliximab clearance. Serum albumin

levels in our study populations ranged from 26.3 to 50.1 g/L, resulting in a decrease in infliximab CL by 47% from 0.45 to 0.24 L/day for a patient with median body weight and non-positive ATI status. A significant effect of serum albumin on infliximab clearance has also been reported in patients with inflammatory bowel disease [28-30]. Albumin is an endogenous protein that, like therapeutic IgGs such as infliximab, is protected from catabolism by binding to the Brambell receptor, FcRn, resulting in antibody salvage and recirculation [31]. A high serum baseline albumin may be the result of a low clearance of albumin and antibodies by efficient FcRn-mediated protein salvage. Baseline serum albumin concentration being a significant covariate suggests that albumin homeostasis can be regarded as a surrogate marker for that of therapeutic IgGs in our sarcoidosis population.

During the forward inclusion procedure, an association between covariate inflammatory biomarker sIL-2R and infliximab clearance was found, with high baseline sIL-2R being associated with high clearance. Interleukin-2 is a pro-inflammatory cytokine secreted by activated T lymphocytes. It is thought to play a key role in sarcoidosis as it stimulates proliferation of T lymphocytes by binding to the IL-2R. This receptor can be released in a soluble form, sIL-2R, which has been proposed as a marker for sarcoidosis disease activity [32-35]. In rheumatoid arthritis and inflammatory bowel disease inflammatory biomarker CRP has been associated with infliximab trough concentrations and infliximab clearance, likely due to effect on target-mediated drug disposition, a mechanism of elimination frequently reported for monoclonal antibodies [8-12]. Because CRP is not regarded as an adequate biomarker to reflect inflammatory disease activity in sarcoidosis we included biomarkers that do represent inflammatory activity in sarcoidosis (sIL-2R, ACE and SUVmax on ^{18}F-FDG PET scan) as potential indirect markers for TNF burden in our population. Although, the association between baseline sIL-2R and CL was not maintained when the backward elimination procedure was performed, this finding suggests that TNF burden at baseline may also play a role in clearance of infliximab in sarcoidosis.

Additionally, Ternant *et al.* [12] reported infliximab pharmacokinetics to be influenced by the *FCGR3A* gene, which encodes FcγRIIIA, a receptor for the Fc portion of IgG that is expressed on macrophages and natural killer cells. The single nucleotide polymorphism *FCGR3A* -158V/F, which results in a different receptor affinity for human IgG, has been associated with infliximab clearance and also response to infliximab treatment. Homozygous V/V patients have a higher affinity for human IgG, eliminating infliximab faster and being more sensitive to treatment in Crohn's disease [36-38]. However, in our study we could not replicate the association between the single nucleotide polymorphism *FCGR3A* -158V/F and infliximab pharmacokinetics in sarcoidosis patients. Response to infliximab in sarcoidosis has been associated with the single nucleotide polymorphism *TNF* -308 G/A [39]. This functional SNP is located in the promotor region of the gene and has been related to differential gene expression [40,41]. Variability in this SNP as a predictor for infliximab pharmacokinetics has not been studied before. We hypothesized that a higher TNF expression could potentially result in a higher infliximab clearance. However, we did not find a significant correlation with infliximab pharmacokinetics.

In patients positive for ATIs mean CL was higher compared to patients who tested negative for ATIs or in whom ATIs were not assessed because there was no clinical suspicion of development of ATIs. Mean CL in ATI positive patients was 81% higher compared to the other patients. The bootstrap analysis showed a wide 95% confidence interval for this covariate effect. Likely this is due to the small number of patients positive for ATIs.

Systemic infliximab clearance in sarcoidosis was comparable to earlier reported values in ankylosing spondylitis and rheumatoid arthritis, and slightly lower compared to Crohn's disease and ulcerative colitis [15-17,28,29,42-44]. A potential explanation for this finding is that in inflammatory bowel disease, infliximab also disappears from the circulation through intestinal protein loss [45], resulting in a larger estimated clearance value. Another potential explanation could be a different TNF burden and therefore a difference in target-mediated clearance [11,46].

We found a large variability in V2 compared with CL and V1, possibly because of the lack of multiple measurements during the distribution phase. This phenomenon has been reported previously [28]. BW and BSA being covariates on CL and V1, respectively, has also been reported before [15-17].

In rheumatoid arthritis and Crohn's disease the addition of low dose concomitant immunosuppressive medication has been previously reported to influence infliximab clearance [11,28]. In our study all patients received low dose concomitant immunosuppressive medication during the study period. Therefore, we were not able to study this as a covariate.

The pharmacodynamic analysis showed a significant association between AUC of infliximab and change in FVC, with a worse outcome in patients with a higher AUC. In a previous analysis on this study population, we described a predictive relationship between baseline SUVmax of the lung and change in FVC [6]. When the baseline SUVmax value of the lung was added to the correlation between AUC and change in FVC, the relationship between these variables was no longer statistically significant. A potential explanation for this finding may be that patients with a low SUVmax of the lung at baseline have little inflammation, and therefore do not have much room for improvement. This low inflammation burden means low TNF levels and because infliximab is only detected when not bound to TNF, will result in higher levels of unbound and thus detectable infliximab.

Median infliximab trough concentrations were higher compared to earlier reports on other inflammatory diseases. This is not surprising, because the interval between infliximab doses in sarcoidosis treatment is shorter compared to other treatment indications. No therapeutic window for infliximab trough levels is known in sarcoidosis. However, taking into account the absence of an exposure-response relationship found in our study, these trough levels are considered supratherapeutic, indicating overdosing in the current treatment protocol. The developed pharmacokinetic model is essential in finding the optimal infliximab dosing regimen in severe sarcoidosis.

In conclusion, baseline BSA, BW, serum albumin and ATI status were found to influence the pharmacokinetics of infliximab pharmacokinetics in severe sarcoidosis. No exposure-response relationship was found, indicating overdosing in the current treatment protocol.

ACKNOWLEDGEMENTS

We are grateful to Kees de Bruijn, Lilian Tebeest and Annette van der Vis for their expert contribution in the measurement of infliximab serum levels and genotyping.

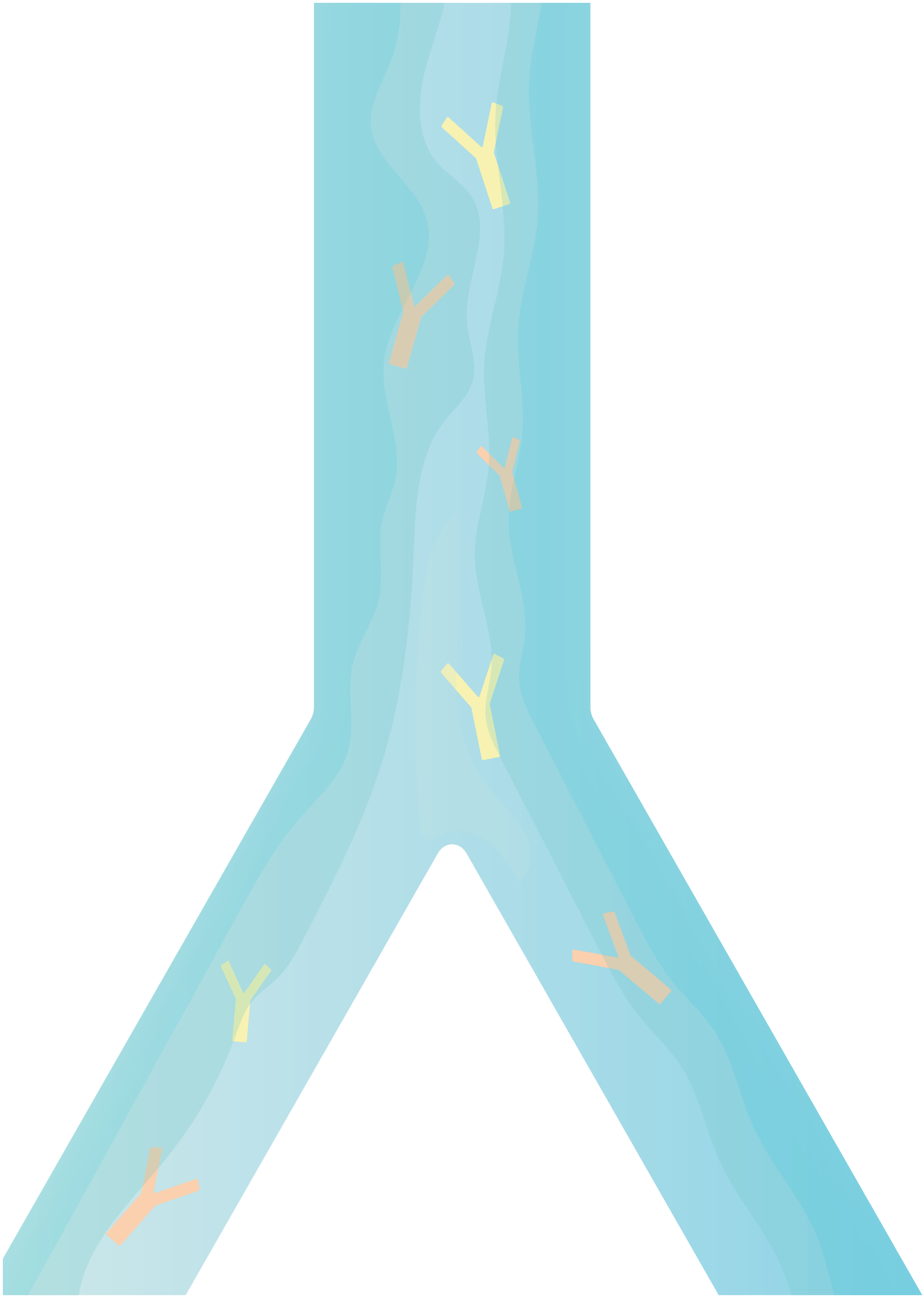
REFERENCES

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
2. Ziegenhagen MW, Rothe ME, Zissel G, Muller-Quernheim J. Exaggerated TNFalpha release of alveolar macrophages in corticosteroid resistant sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2002; 19: 185-190.
3. Vorselaars AD, Wuyts WA, Vorselaars VM, Zanen P, Deneer VH, Veltkamp M, Thomeer M, van Moorsel CH, Grutters JC. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* 2013; 144: 805-812.
4. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. *Arch Intern Med* 1995; 155: 846-851.
5. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
6. Vorselaars AD, Crommelin HA, Deneer VH, Meek B, Claessen AM, Keijsers RG, van Moorsel CH, Grutters JC. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J* 2015; 46: 175-185.
7. Centocor. Remicade(R) (infliximab), prescribing information. 2006.
8. Brandse JF, Mathot RA, van der Kleij D, Rispens T, Ashruf Y, Jansen JM, Rietdijk S, Lowenberg M, Ponsioen CY, Singh S, van den Brink GR, D'Haens GR. Pharmacokinetic Features and Presence of Antidrug Antibodies Associate With Response to Infliximab Induction Therapy in Patients With Moderate to Severe Ulcerative Colitis. *Clin Gastroenterol Hepatol* 2016; 14: 251-258.
9. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. *Arthritis Rheum* 2006; 54: 3782-3789.
10. Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, Nurmohamed MT, Tak PP, Dijkmans BA, Aarden L. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 704-707.
11. Ternant D, Ducourau E, Perdriger A, Corondan A, Le Goff B, Devauchelle-Pensec V, Solau-Gervais E, Watier H, Goupille P, Paintaud G, Mulleman D. Relationship between inflammation and infliximab pharmacokinetics in rheumatoid arthritis. *Br J Clin Pharmacol* 2014; 78: 118-128.
12. Ternant D, Berkane Z, Picon L, Gouilleux-Gruart V, Colombel JF, Allez M, Louis E, Paintaud G. Assessment of the Influence of Inflammation and FCGR3A Genotype on Infliximab Pharmacokinetics and Time to Relapse in Patients with Crohn's Disease. *Clin Pharmacokinet* 2015; 54: 551-562.
13. Buurman DJ, Maurer JM, Keizer RJ, Kosterink JG, Dijkstra G. Population pharmacokinetics of infliximab in patients with inflammatory bowel disease: potential implications for dosing in clinical practice. *Aliment Pharmacol Ther* 2015; 42: 529-539.
14. Ternant D, Aubourg A, Magdelaine-Beuzelin C, Degenne D, Watier H, Picon L, Paintaud G. Infliximab pharmacokinetics in inflammatory bowel disease patients. *Ther Drug Monit* 2008; 30: 523-529.
15. Xu Z, Seitz K, Fasanmade A, Ford J, Williamson P, Xu W, Davis HM, Zhou H. Population pharmacokinetics of infliximab in patients with ankylosing spondylitis. *J Clin Pharmacol* 2008; 48: 681-695.
16. Ternant D, Mulleman D, Lauferon F, Vignault C, Ducourau E, Wendling D, Goupille P, Paintaud G. Influence of methotrexate on infliximab pharmacokinetics and pharmacodynamics in ankylosing spondylitis. *Br J Clin Pharmacol* 2012; 73: 55-65.
17. Brandse JF, Mould D, Smeekes O, Ashruf Y, Kuin S, Strik A, van den Brink GR, D'Haens GR. A Real-life Population Pharmacokinetic Study Reveals Factors Associated with Clearance and Immunogenicity of Infliximab in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2017; 23: 650-660.
18. Keicho N, Kitamura K, Takaku F, Yotsumoto H. Serum concentration of soluble interleukin-2 receptor as a sensitive

parameter of disease activity in sarcoidosis. *Chest* 1990; 98: 1125-1129.
19. Rothkrantz-Kos S, van Dieijen-Visser MP, Mulder PG, Drent M. Potential usefulness of inflammatory markers to monitor respiratory functional impairment in sarcoidosis. *Clin Chem* 2003; 49: 1510-1517.
20. Miyoshi S, Hamada H, Kadowaki T, Hamaguchi N, Ito R, Irifune K, Higaki J. Comparative evaluation of serum markers in pulmonary sarcoidosis. *Chest* 2010; 137: 1391-1397.
21. Keijsers RG, van den Heuvel DA, Grutters JC. Imaging the inflammatory activity of sarcoidosis. *Eur Respir J* 2013; 41: 743-751.
22. Keir G, Wells AU. Assessing pulmonary disease and response to therapy: which test? *Semin Respir Crit Care Med* 2010; 31: 409-418.
23. Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT, Stapel S, Tak PP, Aarden L, Dijkmans B. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54: 711-715.
24. Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. *Clin Pharmacokinet* 2005; 44: 1051-1065.
25. Verwoerd A, Vorselaars AD, van Moorsel CH, Bos WJ, van Velzen-Blad H, Grutters JC. Discrepant elevation of sIL-2R levels in sarcoidosis patients with renal insufficiency. *Eur Respir J* 2015; 46: 277-280.
26. Keizer RJ, Karlsson MO, Hooker A. Modeling and Simulation Workbench for NONMEM: Tutorial on Pirana, PsN, and Xpose. *CPT Pharmacometrics Syst Pharmacol* 2013; 2: e50.
27. Proost JH. Combined proportional and additive residual error models in population pharmacokinetic modelling. *Eur J Pharm Sci* 2017.
28. Fasanmade AA, Adedokun OJ, Blank M, Zhou H, Davis HM. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. *Clin Ther* 2011; 33: 946-964.
29. Fasanmade AA, Adedokun OJ, Ford J, Hernandez D, Johanns J, Hu C, Davis HM, Zhou H. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. *Eur J Clin Pharmacol* 2009; 65: 1211-1228.
30. Fasanmade AA, Adedokun OJ, Olson A, Strauss R, Davis HM. Serum albumin concentration: a predictive factor of infliximab pharmacokinetics and clinical response in patients with ulcerative colitis. *Int J Clin Pharmacol Ther* 2010; 48: 297-308.
31. Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. *Clin Pharmacol Ther* 2012; 91: 635-646.
32. Hunninghake GW, Bedell GN, Zavala DC, Monick M, Brady M. Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis. *Am Rev Respir Dis* 1983; 128: 634-638.
33. Lawrence EC, Brousseau KP, Berger MB, Kurman CC, Marcon L, Nelson DL. Elevated concentrations of soluble interleukin-2 receptors in serum samples and bronchoalveolar lavage fluids in active sarcoidosis. *Am Rev Respir Dis* 1988; 137: 759-764.
34. Rubin LA, Kurman CC, Fritz ME, Biddison WE, Boutin B, Yarchoan R, Nelson DL. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J Immunol* 1985; 135: 3172-3177.
35. Grutters J, Fellrath J, Mulder L, Janssen R, van den Bosch,Jules M M, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest* 2003; 124: 186-195.
36. Louis E, El Ghoul Z, Vermeire S, Dall'Ozzo S, Rutgeerts P, Paintaud G, Belaiche J, De Vos M, Van Gossum A, Colombel JF, Watier H. Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease. *Aliment Pharmacol Ther* 2004; 19: 511-519.
37. Louis EJ, Watier HE, Schreiber S, Hampe J, Taillard F, Olson A, Thorne N, Zhang H, Colombel JF. Polymorphism in IgG Fc receptor gene FCGR3A and response to infliximab in Crohn's disease: a subanalysis of the ACCENT I study. *Pharmacogenet Genomics* 2006; 16: 911-914.
38. Moroi R, Endo K, Kinouchi Y, Shiga H, Kakuta Y, Kuroha M, Kanazawa Y, Shimodaira Y, Horiuchi T, Takahashi S, Shimosegawa T. FCGR3A-158 polymorphism influences the biological response to infliximab in Crohn's disease through

affecting the ADCC activity. *Immunogenetics* 2013; 65: 265-271.

39. Wijnen PA, Cremers JP, Nelemans PJ, Erckens RJ, Hoitsma E, Jansen TL, Bekers O, Drent M. Association of the TNF-alpha G-308A polymorphism with TNF-inhibitor response in sarcoidosis. *Eur Respir J* 2014; 43: 1730-1739.
40. Hellmig S, Fischbach W, Goebeler-Kolve ME, Folsch UR, Hampe J, Schreiber S. A functional promotor polymorphism of TNF-alpha is associated with primary gastric B-Cell lymphoma. *Am J Gastroenterol* 2005; 100: 2644-2649.
41. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci U S A* 1997; 94: 3195-3199.
42. Dotan I, Ron Y, Yanai H, Becker S, Fishman S, Yahav L, Ben Yehoyada M, Mould DR. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis* 2014; 20: 2247-2259.
43. Aubourg A, Picon L, Lecomte T, Bejan Angoulvant T, Paintaud G, Ternant D. A robust estimation of infliximab pharmacokinetic parameters in Crohn's disease. *Eur J Clin Pharmacol* 2015; 71: 1541-1542.
44. Kavanaugh A, St Clair EW, McCune WJ, Braakman T, Lipsky P. Chimeric anti-tumor necrosis factor-alpha monoclonal antibody treatment of patients with rheumatoid arthritis receiving methotrexate therapy. *J Rheumatol* 2000; 27: 841-850.
45. Brandse JF, van den Brink GR, Wildenberg ME, van der Kleij D, Rispens T, Jansen JM, Mathot RA, Ponsioen CY, Lowenberg M, D'Haens GR. Loss of Infliximab Into Feces Is Associated With Lack of Response to Therapy in Patients With Severe Ulcerative Colitis. *Gastroenterology* 2015; 149: 350-355.
46. Passot C, Mulleman D, Bejan-Angoulvant T, Aubourg A, Willot S, Lecomte T, Picon L, Goupille P, Paintaud G, Ternant D. The underlying inflammatory chronic disease influences infliximab pharmacokinetics. *MAbs* 2016; 8: 1407-1416.



8

Summary and general discussion

SUMMARY

The objective of this thesis was to investigate the effectiveness of anti-tumor necrosis factor (TNF) agents infliximab and adalimumab in severe sarcoidosis and to assess whether pharmacogenetics, inflammatory biomarkers and pharmacokinetics and pharmacodynamics can aid in more precision based anti-TNF treatment in severe sarcoidosis.

In **chapter 2** we provide an overview of the current knowledge of anti-TNF therapeutics in sarcoidosis, especially highlighting the differences in effectiveness. TNF is a critical factor in the development and progression of sarcoidosis. It plays a pivotal role in the formation of granulomas. Therefore, inhibiting the effect of TNF by immunomodulatory therapy is a rational proposition. There are two types of anti-TNF agents used in sarcoidosis. First, there are the non-targeted immunomodulatory drugs with an inhibitory effect on TNF production. Examples are thalidomide, pentoxifylline and apremilast. They are not regularly prescribed in clinical practice because of an unfavorable risk/benefit ratio. The second type is the group of targeted anti-TNF biologicals, specifically inhibiting TNF, such as infliximab, adalimumab and etanercept. These drugs are given when first- and second-line treatment options have failed. Infliximab and adalimumab have shown to be of benefit in sarcoidosis patients; etanercept was found to be insufficiently effective. This difference may be explained by the lower affinity of etanercept for membrane-bound TNF, etanercept being unable to induce cellular apoptosis and variation in expression of certain immune-related genes. Apart from their beneficial effects, severe side effects have been reported, including infections and infusion reactions. Moreover, patients have to be closely monitored for possible development of antibodies against infliximab during treatment and return of disease activity after treatment cessation. There is a clinical need for evidence based guidelines describing when and how to prescribe anti-TNF therapeutics in sarcoidosis and for identification of putative responsive patients.

Chapter 3 reports on a prospective, single arm study in 56 patients with severe sarcoidosis, refractory to first- and second-line treatment, who were treated with infliximab in a dosing regimen of 5 mg/kg at week 0, 2 and every four weeks thereafter. After *six months* of treatment, response was evaluated for three domains: organ function, inflammatory biomarkers and quality of life. Also, infliximab trough levels in serum were measured. Mean improvement in forced vital capacity (FVC) was 6.6% predicted (p=0.0007), whereas in the six months before start of treatment, lung function deteriorated. Maximum standardized uptake value (SUVmax) of pulmonary parenchyma on ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET) scan decreased by 3.93 (p<0.0001). High SUVmax of pulmonary parenchyma at baseline predicted FVC improvement (R=0.62, p=0.0004). Mean patient global assessment score on a visual analogue scale showed a clinically significant decrease of -14.6 after six months of treatment. Mean physical functioning score on the Short-form 36 increased by 8.2 after six months of treatment. The mean infliximab trough serum level was 18 µg/mL and no correlation between infliximab trough serum

level and response was found. This first prospective open label study showed that infliximab is effective in selected patients with refractory disease and evidence of persistent disease activity.

In **Chapter 4** effectiveness of infliximab in *long-term* treatment was evaluated. A total of 37 patients continued treatment for at least one year and 26 patients further continued infliximab treatment for at least two years. Organ function and inflammatory biomarkers were evaluated every six and three months, respectively, for up to two years. After the first six months of treatment the mean improvement in FVC was 8% predicted and the mean improvement in diffusing capacity of the lung for carbon monoxide corrected for hemoglobin (DLCOc) was 6% predicted. Pulmonary function tests remained stable during the rest of the study period. The mean levels of the inflammatory biomarker soluble interleukin-2 receptor (sIL-2R) decreased from 8721 to 4584 pg/mL in the first three months of treatment and remained rather stable during the rest of the study period. Mean angiotensin-converting enzyme (ACE) also decreased during the first three months of infliximab treatment from 93 to 56 U/L and remained stable during the rest of the study period. We observed that the positive effect of six months treatment with infliximab in severe refractory sarcoidosis can be maintained for at least two years under prolonged treatment. In case of clinical stability and low levels for inflammatory parameters, the dosing interval was lengthened based upon physician's judgement. In patients where the dosing interval was lengthened (n=14), organ function and inflammatory biomarkers remained stable. These data show that the dosing interval between infusions can be safely lengthened in selected patients with clinical stability and low levels of inflammatory markers.

Chapter 5 investigates whether adalimumab could achieve stabilization or improvement of the disease in patients who initially responded to infliximab therapy but had become intolerant to infliximab, typically because of loss of response and infusion reactions because of the formation of antibodies against infliximab. A total of 18 patients switched to adalimumab because of intolerance to infliximab. Organ function improved in seven patients (39%), was stable in six patients (33%) and worsened in five patients (28%) after twelve months of treatment or after six months, if evaluation after twelve months was not available (n=4). In none of the patients biomarker levels of sIL-2R increased. The most reported adverse event was infection (n=10). The findings show that 72% of the patients showed at least a stable disease pattern when they switched from infliximab to adalimumab. Therefore, adalimumab appears to be a suitable alternative in patients who have developed intolerance to infliximab.

In **Chapter 6** we investigated whether variants in the genes encoding TNF, TNF receptors, Fcγ-receptors and HLA genotypes were associated with change in clinical and inflammatory parameters in severe sarcoidosis patients treated with infliximab. We found that the mean improvement of DLCOc was lower in carriers of the *TNFRSF1A* rs1800693 G allele (GG+GA) than in non-carriers (AA) (0.5 vs 6.1% predicted, p=0.001). Furthermore, reduction in sIL-2R and ACE was lower in *TNFRSF1A* rs1800693 G allele carriers than in non-carriers (2473 vs 6273 pg/mL, p=0.004 and 16 vs 38 U/L, p=0.03, respectively). Regarding *TNFRSF1B* T196G, carriers of the T allele had a mean decrease in ACE of 25 U/L versus an increase of 15 U/L in non-carriers of the

T allele (p=0.02). Finally, HLA-DRB*0301 negative patients (tag SNP rs2040410 GG) showed a significantly higher reduction in sIL-2R compared to HLA-DRB1*0301 positive patients (tag SNP rs2040410 carriers of the A allele) (3996 versus 2283 pg/mL, p=0.03). These findings require further confirmation in other studies.

In **Chapter 7** we present a population pharmacokinetic model of infliximab in severe sarcoidosis. In 68 patients peak concentrations, concentrations two weeks after dose administration and trough concentrations were used to develop a two-compartment population pharmacokinetic model using NONMEM. Population pharmacokinetic estimates (typical value (relative standard error)) in the final covariate model were clearance (CL) 0.276 L/day (3.2%), volume of central compartment (V1) 3.16 L (1.6%), intercompartmental clearance (Q) 0.177 L/day (21%) and volume of peripheral compartment (V2) 1.49 L (11%). These population estimates of the pharmacokinetic parameters were comparable to earlier published population pharmacokinetic models in rheumatoid arthritis and ankylosing spondylitis. The population estimate for the clearance of infliximab in sarcoidosis was slightly lower compared to population estimates for the clearance in inflammatory bowel disease. Covariate analyses showed that V1 increased with baseline body surface area and CL increased with positive antibodies against infliximab status, low baseline serum albumin and high body weight. No association with inflammatory activity or genotype and no exposure-response relationship were found.

GENERAL DISCUSSION

Sarcoidosis is a granulomatous disease of unknown cause with a wide heterogeneity in organ manifestation, severity and clinical course. Pulmonary involvement is present in 90% of the patients, but lymph nodes, eyes and skin are often involved as well. Therefore, multiple specialists may be involved in care for sarcoidosis patients [1].

Although the exact etiology of sarcoidosis remains unknown, *in vitro* research has shown that TNF plays a pivotal role in the formation and maintenance of granulomas. This has fueled the hypothesis that anti-TNF agents could be of benefit in the treatment of sarcoidosis [2].

Because most sarcoidosis patients show self-limiting disease or can adequately be treated with first- or second-line medications, only a small number of patients require therapy with anti-TNF agents. Case reports and retrospective series have reported benefit in various manifestations of sarcoidosis, including pulmonary, cutaneous, and ocular manifestations. However, only one prospective study in a (relatively) large group of patients has been performed [3]. Thus current recommendations for the treatment of sarcoidosis are mainly based on expert opinion and 'eminence'-based medicine and often derived from extrapolations from other chronic immune-mediated inflammatory diseases. More insight is needed in the details of anti-TNF therapy in sarcoidosis - especially prospective studies and studies on long-term treatment outcome - to determine effectiveness and assess which patients will benefit most. Research on how to

individualize infliximab treatment in sarcoidosis is far behind compared to other immune-mediated inflammatory diseases such as rheumatoid arthritis, Crohn's disease and ulcerative colitis, especially in the areas of pharmacogenetics, pharmacokinetics and pharmacodynamics.

Effectiveness of infliximab in refractory sarcoidosis

Retrospective case series and case reports on infliximab in sarcoidosis have reported a benefit, but the largest randomized controlled trial by Baughman *et al.* [3] only found a small improvement in pulmonary function with unclear clinical relevance. This study included 90 patients in the treatment group and 44 patients in the placebo group. A mean increase in FVC of 2.5% predicted in the treatment group and no change in the placebo group was reported. These findings differ from the results of our prospective, single arm study described in **chapter 3**. In our population FVC showed a mean improvement of 6.6% predicted after a six months treatment period with infliximab while in the six months prior to infliximab initiation FVC deteriorated. Likely, the different patient selection process causes this different treatment outcome, with a much higher disease activity in our population compared to the population in the study by Baughman *et al.* [3]. One of the inclusion criteria in the study by Baughman *et al.* [3] was that patients should be on a stable dose of prednisone before study entry. In a *post hoc* analysis infliximab therapy was more beneficial in patients with more severe disease. In our study, levels of inflammatory biomarkers, i.e. sIL-2R, ACE and activity on ^{18}F-FDG PET scan, at baseline were relatively high. The only available biomarker in the previous study was ACE, which was within normal range. In our study, inflammatory activity of the pulmonary parenchyma measured by ^{18}F-FDG PET scan at baseline correlated with pulmonary function improvement after six months of infliximab treatment. Because infliximab is an anti-inflammatory agent it will have the highest impact in patients with active inflammatory disease [4]. Our data further showed improvement in extrapulmonary manifestations of sarcoidosis, reduction of inflammatory biomarkers sIL-2R and ACE and also reduction of activity measured with ^{18}F-FDG PET scan. In quality of life questionnaires we observed an improvement on the visual analogue scale score and the physical functioning score of the short form-36. Our study demonstrated that infliximab is beneficial in sarcoidosis refractory to first- and second-line therapy treatment and should especially be considered in patients with evidence of active lung disease.

Above results concern short-term effectiveness of infliximab after six months of treatment. A recent study reported that discontinuation of infliximab after a mean duration of infliximab treatment of 8.5 months resulted in relapse in the majority of patients and therefore physicians are hesitant to discontinue infliximab treatment in responding patients [5]. However, there remains a lack of well-documented evidence on whether response is maintained if infliximab infusions are continued. Small studies have reported benefit after more than six months of infliximab treatment but none have reported multiple moments of follow-up to investigate whether response after six months is actually maintained or improvement continues or diminishes over time. Study results in **chapter 4** show that response after six months is maintained for at least two years

when treatment is continued. Moreover, in patients who are treated for a longer period of time guidelines recommend to gradually withdraw the drug by increasing the interval between doses [6]. Our study showed that when lengthening the interval between infliximab doses from four to six weeks organ function and inflammatory biomarkers levels remain stable. This indicates that either these subjects have finally entered a spontaneous remission state of their disease or only need small amounts of infliximab to maintain disease control. This is the first study to provide clinical data to support the recommendation to taper infliximab. Whether the dosing interval can be further lengthened to eight weeks or longer and when or if infliximab can be safely discontinued requires further studies.

Adalimumab in patients who have developed intolerance to infliximab

Chapter 4 demonstrates that some patients lose response to infliximab because they develop antibodies against infliximab. Besides loss of response, infusion reactions often occur in these patients. Thus patients develop intolerance to infliximab. In clinical practice these patients often switch to adalimumab [7]. Moreover, patients suffering from other side effects of infliximab that have been reported less frequently in adalimumab treatment, such as pericarditis, could switch. Usually the goal of treatment is improvement, but because these patients have been treated with another anti-TNF agent just prior to start of adalimumab, the objective of adalimumab treatment is to at least maintain stability. Our findings in **chapter 5** show that 72% of patients at least remained stable when they switched from infliximab to adalimumab, making adalimumab a suitable alternative for infliximab in these patients.

Although the study design was not intended to compare effectiveness of adalimumab with that of infliximab, our results do not suggest adalimumab to be more effective than infliximab. This is in line with previous studies on adalimumab in sarcoidosis and current guidelines for clinical practice. They recommend the use of infliximab as the preferred specific anti-TNF agent in severe sarcoidosis.

Role of genetic variations in the effectiveness of infliximab in sarcoidosis

Response to infliximab has shown a great variability: in **chapter 3** 21% of patients showed a partial or no response. Preferably only patients who are likely to respond should be treated with infliximab because of potential side effects and high costs of the medication. Side effects of infliximab are infections, including tuberculosis, infusion reactions and further deterioration of cardiac failure. Also, infliximab is an expensive medication. Therefore, there is a need for studying factors that may predict the response.

In **chapter 3** we reported a positive correlation between the SUVmax value at baseline on ^{18}F-FDG PET scan and improvement in pulmonary function upon infliximab treatment. In rheumatoid arthritis and inflammatory bowel disease many studies focused on which factors predict response. One of the areas of interest has been pharmacogenetics. Genes of interest included those encoding TNF, TNFR1, TNFR2, and Fcγ-receptor. In **chapter 6** we investigated whether

genetic variations in these genes were associated with the response to infliximab in pulmonary function and inflammatory biomarkers. We found that carriers of the *TNFRSF1A* rs1800693 G allele had a significantly lower improvement in DLCOc and also a lower reduction in sIL-2R and ACE after six months compared with non-carriers (AA). Interestingly, this gene has been studied in inflammatory bowel disease and rheumatoid arthritis but interest subsided because of lack of association between *TNFRSF1A* genetic variants and response to infliximab [8], suggesting a difference in pathophysiology between sarcoidosis and other immune-mediated inflammatory diseases. Our findings require further confirmation in other studies. If replicated, our findings may aid in clinical decision-making in sarcoidosis.

Pharmacokinetics and exposure-response relationship of infliximab in sarcoidosis

Another area of research that is far behind in sarcoidosis compared to rheumatoid arthritis and inflammatory bowel disease is knowledge of the pharmacokinetics and pharmacodynamics of infliximab. Knowledge in this area could be of great assistance in optimizing infliximab treatment: information on pharmacokinetics of infliximab could aid in determining the optimal dosing regimen in sarcoidosis. The currently recommended maintenance dosing regimen differs from registered dosing regimens for other immune-mediated inflammatory diseases: in rheumatoid arthritis the approved maintenance dose is 3 mg/kg every eight weeks, whereas in ankylosing spondylitis, 5 mg/kg every six weeks is approved and in inflammatory bowel disease 5 mg/kg every eight weeks [9]. In sarcoidosis the recommended maintenance dosing regimen is 5 mg/kg every four weeks [6]. This intensive dosing regimen in sarcoidosis is primarily based on expert opinion. Knowledge on the pharmacokinetic profile of infliximab in sarcoidosis patients could aid in designing a more evidence based and potentially personalized dosing regimen.

We studied pharmacokinetics in 68 patients during infliximab treatment with a maximum follow-up of two years. Peak (one hour after the end of infusion) and trough (just before the following dose) levels and levels two weeks after dosing were drawn throughout the study period. Using NONMEM we estimated parameters for distribution and clearance and we tested whether covariates influenced these parameters significantly. Significant covariates influencing clearance were body weight, antibodies against infliximab and serum albumin. The volume of the central compartment was significantly influenced by body surface area. Interestingly, during the forward inclusion step sIL-2R was found to be a significant covariate for clearance. Although this covariate lost significance during the backward elimination procedure, it is an interesting finding. The influence of sIL-2R on clearance suggests an important role for target-mediated clearance of infliximab in sarcoidosis. This supports our hypothesis that in patients with more inflammation as indicated by serum biomarkers and ^{18}F-FDG PET scan, more target for infliximab, i.e. TNF, is present. Estimates for volume of the central compartment, intercompartmental clearance and volume of the peripheral compartment were comparable to previous reports in rheumatoid arthritis. The population estimate for the clearance of infliximab in sarcoidosis was slightly lower compared to populations estimated for the clearance in inflammatory bowel disease.

We also studied whether exposure to infliximab was associated with response. We did find a statistically significant correlation between exposure to infliximab and change in FVC. A high exposure was associated with a lower response. When inflammation was taken into account by correcting for baseline SUVmax of the pulmonary parenchyma on the ^{18}F-FDG PET scan, the relationship was no longer significant. A potential explanation for this finding may be that patients with a low SUVmax of the lung at baseline have little inflammation. This low inflammation burden means low TNF levels. Because infliximab is only detected in free form not bound to TNF a low inflammatory burden will result in a higher level of unbound and thus detectable infliximab.

The median infliximab trough concentration was 18 µg/mL, which is higher compared to earlier reports on rheumatoid arthritis and inflammatory bowel disease [10,11]. This is expected, because the interval between infliximab doses in sarcoidosis treatment is shorter compared to other treatment indications and the dose equal or higher than in these other immune-mediated inflammatory diseases.

We were unable to determine a therapeutic window for infliximab in sarcoidosis. No association between trough level and response score or exposure and response was found. This could be because the levels are supratherapeutic meaning that there might be room for dose reduction in clinical practice. Another explanation is that there simply is no therapeutic window for infliximab in sarcoidosis and blood levels of infliximab are not related to response.

FUTURE PERSPECTIVES AND CONCLUDING REMARKS

This thesis has addressed multiple aspects of anti-TNF treatment in sarcoidosis with the aim to increase the evidence for recommendations to treat patients. We have identified new areas of interest that in the future may help in further optimizing and/or personalizing pharmacotherapy in severe sarcoidosis.

Targeted anti-TNF agents have emerged as a completely new line of treatment in severe sarcoidosis. One of the hallmarks of sarcoidosis is the fact that it is an orphan disease. Traditionally, the introduction of new, clinically validated therapies for orphan diseases lags behind diseases with larger patient populations. The underlying reason is the low potential economic benefit for pharmaceutical companies and the challenge to run clinical trials with sufficient sample sizes. Considering this is a given fact, the sarcoidosis field should closely follow developments in other diseases with larger patient populations that closest resemble the presumed pathogenesis of sarcoidosis.

In the following sections several aspects that require further action based on findings in this thesis are discussed.

Recommendations for daily practice

This thesis shows that infliximab therapy is effective in selected patients with severe refractory sarcoidosis and evidence of persistent inflammatory activity. Patient selection should therefore ideally be based on criteria for both disease severity *and* inflammatory activity. Further we found that when infliximab treatment is continued, response is maintained. A previous study demonstrated that when infliximab is discontinued, the majority of patients experience disease relapse [5]. Our current recommendation therefore is to continue treatment when a patient shows a positive response.

We also observed that already at three months after initiation of infliximab, the inflammatory biomarker sIL-2R significantly decreased. In clinical practice response is evaluated after six months of treatment. Our results suggest that whether a patient responds to infliximab treatment or not may already be obvious after three months of treatment. In patients unresponsive to infliximab this would save months of treatment and being at risk for adverse events without benefit. However, the optimal moment to evaluate response could differ between disease phenotypes and should be subject of further research.

Furthermore, there is a great clinical need to know how long patients should be treated with infliximab and how discontinuation should occur. General opinion is to gradually withdraw the drug by increasing the interval between doses, but there are no clinical trials comparing relapse rate after discontinuation preceded by tapering with discontinuation without tapering [6].

In patients that responded to infliximab therapy but developed intolerance to infliximab, our results support and provide evidence for the recommendation to switch to adalimumab in these cases.

Optimizing treatment: room for dose reduction?

Importantly, our research did not reveal a significant exposure-response relationship for infliximab in sarcoidosis on the basis of the currently used treatment protocol. Moreover, we found that when the dosing interval was lengthened and the infliximab trough concentration decreased to values as low as 2 µg/mL, the response was maintained and no deterioration was observed. Interestingly, the mean trough concentration found in our population was 18 µg/mL. In inflammatory bowel disease currently a therapeutic window for the trough concentration of 3 to 7 µg/mL is being used in clinical practice for determining the optimal dose and dosing interval, especially in patients who do not respond or who lose response [12]. Recent new insights in inflammatory bowel disease have suggested that in order to achieve complete response on both clinical and biomarker endpoints target infliximab trough levels potentially should be higher, namely 6 to 10 µg/mL. Others have suggested that there may not be one therapeutic window for infliximab for the entire population in inflammatory bowel disease patients, but that the concentration required for effect is specific for disease phenotype or pathophysiology. This would require disease stratification [13-15]. Taken altogether, these findings suggest that the infliximab concentrations in sarcoidosis may be supratherapeutic and that in the current treatment protocol we are overdosing. These findings justify lowering the infliximab dosing regimen in a controlled setting.

But, how much should we lower the dose? The results presented in this thesis do not provide us with a target or 'therapeutic window' for infliximab in sarcoidosis. Future studies are needed to establish the minimal effective concentration in severe sarcoidosis treatment. The previously mentioned findings on target concentrations in inflammatory bowel disease seem an obvious starting point.

Predicting response

In this thesis we evaluated potential therapeutic predictive factors in patients with severe, refractory sarcoidosis treated with infliximab. We demonstrated that inflammatory disease activity at baseline and several genetic variations associated with response. A higher disease activity as measured by ^{18}F-FDG PET scan at baseline suggests more TNF and therefore more target for anti-TNF agents. The *TNFRSF1A* rs18000693 genotype was associated with change in clinical and inflammatory parameters. A genetic variation in *TNFRSF1B* and the absence of HLA-DRB*0301 were also associated with differential changes in inflammatory biomarkers.

These are promising biomarkers to help distinguish which patients will benefit most from infliximab treatment. Likely, not one single biomarker will be able to make the differentiation between responders and nonresponders, but rather a combination or panel of multiple biomarkers, clinical parameters and genotypes can really aid in predicting which patients are most likely to respond to infliximab. In the future, our findings may be included in such a panel.

Evaluating response: universal endpoint

Throughout this thesis several endpoints have been used to define response, including pulmonary function tests and inflammatory biomarkers. A gold standard on endpoints in sarcoidosis is currently lacking. This lack of consensus on which outcome measure should be used in clinical trials impedes comparisons between clinical trials. It also hinders an optimal translation from clinical trials to every day practice. In a disease as complex and heterogeneous as sarcoidosis, it is improbable that a single parameter will give a complete image of the response to treatment. In **chapter 3** we propose a composite of organ function, disease activity and quality of life evaluation as a tool to evaluate response. This tool was inspired by the disease activity score-28 (DAS28) that is widely used in rheumatoid arthritis to monitor the disease course and to determine the response to treatment. Future studies are needed to validate the composite score we propose here.

Biosimilars

Biologicals such as infliximab and adalimumab are expensive. Recently, biosimilars, generic versions of biologicals, of infliximab have entered the market and are now widely used in various patient populations. Regulatory approval has been obtained in the EU following European Medicine Agency guidance documents [16]. These guidance documents comprise a thorough comparison of the physicochemical characteristics of the biosimilar and the originator product

in combination with a limited clinical trial program to assess bioequivalence and absence of clinically relevant differences. Clinical comparison studies have not been performed for all treatment indications: the approval may be extrapolated across different treatment indications. No bioequivalence or clinical comparison studies have been reported in sarcoidosis patients. Our population pharmacokinetic model with infliximab/Remicade® can be useful in future studies to assess bioequivalence and to compare pharmacokinetic profiles of biosimilars to those of the originator product, infliximab/Remicade®, in a rare disease such as sarcoidosis.

Alternatives for anti-TNF agents

Biologicals specifically targeting TNF have added a new line to the pharmacotherapeutic arsenal available for the treatment of sarcoidosis and have increased therapeutic options in patients with severe, chronic disease. In other immune-mediated inflammatory diseases progression has been made with the introduction of biologicals specifically targeting other messenger molecules involved in the disease pathology. Because sarcoidosis is a rare disease it is unlikely that medications will be developed by the pharmaceutical industry just for the sarcoidosis patient population. The sarcoidosis field should therefore closely follow progress in the field of other immune-mediated inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. The search for specific drivers that play a key role in sarcoidosis, other than TNF, should continue. One should particularly focus on pathology-driving molecules that can be targeted by agents that are already available (*e.g.* canakinumab). Such agents that have been introduced in the treatment protocols for other immune-mediated inflammatory diseases and are expected to also be of benefit in sarcoidosis patients based on sarcoidosis pathology should then be clinically evaluated in sarcoidosis (such as 'study of efficacy, safety and tolerability of ACZ885 (Canakinumab) in patients with pulmonary sarcoidosis', ClinicalTrials.gov Identifier: NCT02888080). Hopefully, this will lead to monoclonal antibodies with different molecular targets being available for severe sarcoidosis patients based on solid evidence. Together with the expansion of treatment options in severe sarcoidosis, also biomarkers should be developed that determine what is the dominant pathophysiological mechanism in a particular patient and subsequently which agent is most suitable for this patient, rather than using the trial-and-error approach.

CONCLUDING REMARKS

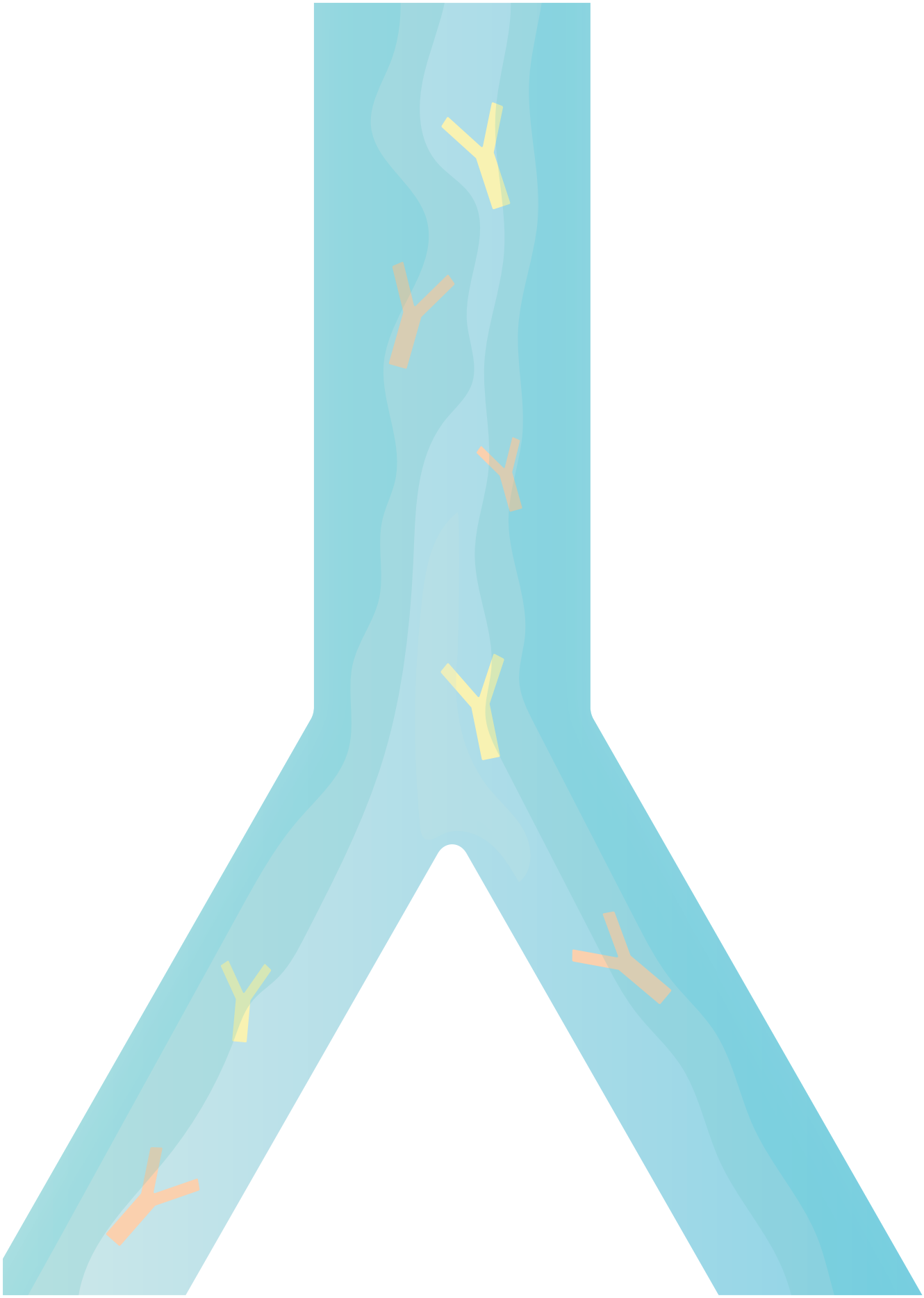
Ideally, in the future health care professionals dealing with sarcoidosis would be able to choose the optimal drug or drug class which will most likely be effective with the least side effects in each individual presenting with severe sarcoidosis. This choice would be based on several patient characteristics including clinical symptoms, radiological findings, disease activity, genotype and biomarkers indicating which immunological pathway(s) is/are most dysfunctional in this particular patient. The optimal dosing regimen will then be calculated based on patient characteristics

of each individual patient, such as body weight, body surface area and serum albumin levels. In addition, for each individual patient the dosing regimen would be further optimized using blood concentration measurements. The dose and/or dosing interval would then be adapted according to evidence based targets.

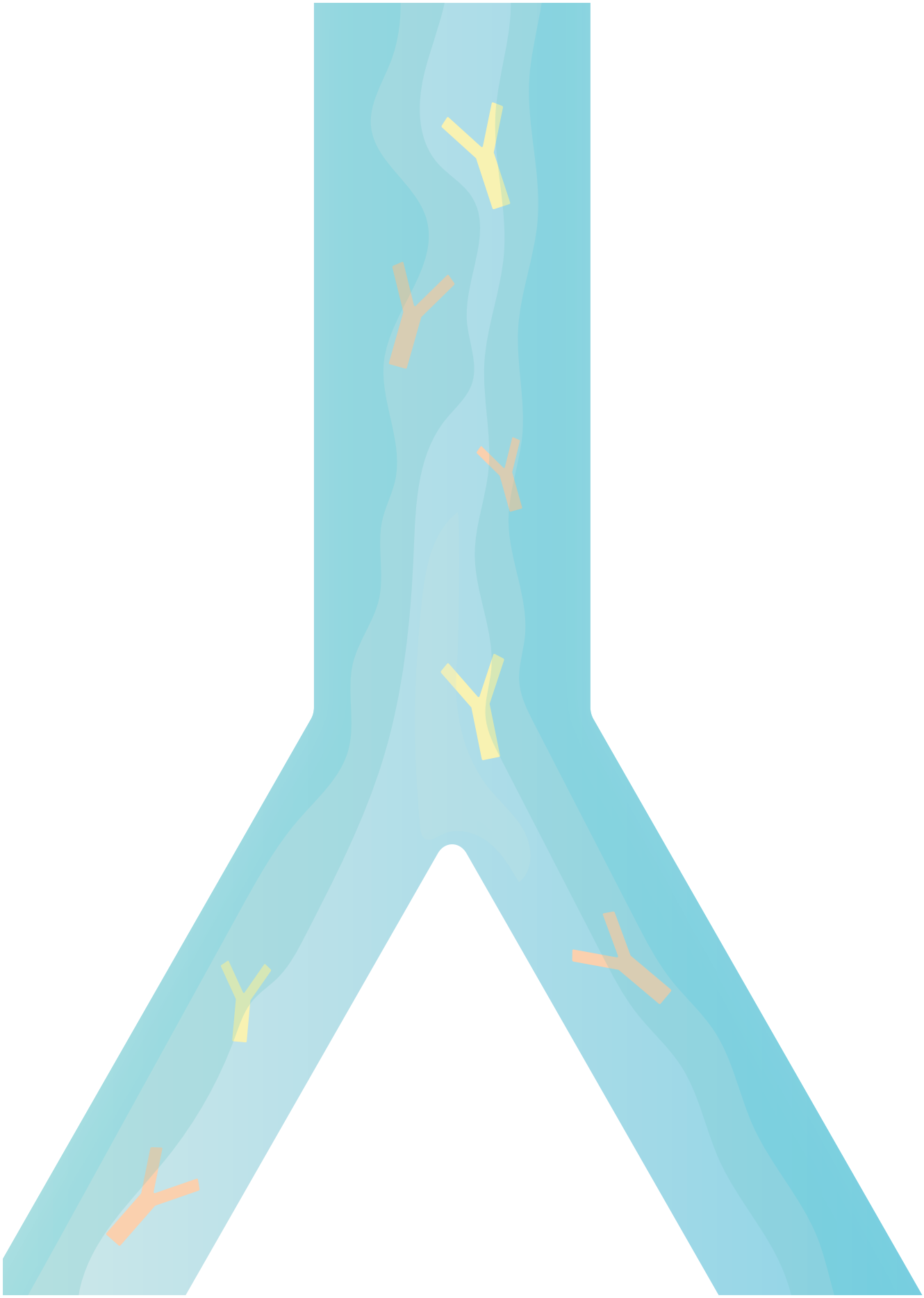
This thesis has taken a first step towards precision medicine in anti-TNF therapy in severe sarcoidosis. Hopefully, its findings will contribute to improvement of patient care in severe sarcoidosis and inspire future researchers on the road to a broader understanding of this enigmatic and fascinating disorder and improvement of its treatment strategies.

REFERENCES

1. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med* 1999; 160: 736-755.
2. Baughman RP, Iannuzzi M. Tumour necrosis factor in sarcoidosis and its potential for targeted therapy. *BioDrugs* 2003; 17: 425-431.
3. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES, Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med* 2006; 174: 795-802.
4. Moller DR. Negative clinical trials in sarcoidosis: failed therapies or flawed study design? *Eur Respir J* 2014; 44: 1123-1126.
5. Vorselaars AD, Verwoerd A, van Moorsel CH, Keijsers RG, Rijkers GT, Grutters JC. Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J* 2014; 43: 602-609.
6. Drent M, Cremers JP, Jansen TL, Baughman RP. Practical eminence and experience-based recommendations for use of TNF-alpha inhibitors in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2014; 31: 91-107.
7. Baughman RP, Lower EE, Kaufman AH. Ocular sarcoidosis. *Semin Respir Crit Care Med* 2010; 31: 452-462.
8. Medrano LM, Taxonera C, Marquez A, Barreiro-de Acosta M, Gomez-Garcia M, Gonzalez-Artacho C, Perez-Calle JL, Bermejo F, Lopez-Sanroman A, Martin Arranz MD, Gisbert JP, Mendoza JL, Martin J, Urcelay E, Nunez C. Role of TNFRSF1B polymorphisms in the response of Crohn's disease patients to infliximab. *Hum Immunol* 2014; 75: 71-75.
9. Centocor. Remicade(R) (infliximab), prescribing information. 2006.
10. Vande Casteele N, Khanna R, Levesque BG, Stitt L, Zou GY, Singh S, Lockton S, Hauenstein S, Ohrmund L, Greenberg GR, Rutgeerts PJ, Gils A, Sandborn WJ, Vermeire S, Feagan BG. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. *Gut* 2015; 64: 1539-1545.
11. van der Maas A, van den Bemt BJ, Wolbink G, van den Hoogen FH, van Riel PL, den Broeder AA. Low infliximab serum trough levels and anti-infliximab antibodies are prevalent in rheumatoid arthritis patients treated with infliximab in daily clinical practice: results of an observational cohort study. *BMC Musculoskelet Disord* 2012; 13:184.
12. Vande Casteele N, Feagan BG, Gils A, Vermeire S, Khanna R, Sandborn WJ, Levesque BG. Therapeutic drug monitoring in inflammatory bowel disease: current state and future perspectives. *Curr Gastroenterol Rep* 2014; 16: 378.
13. Ungar B, Levy I, Yavne Y, Yavzori M, Picard O, Fudim E, Loebstein R, Chowers Y, Eliakim R, Kopylov U, Ben-Horin S. Optimizing Anti-TNF-alpha Therapy: Serum Levels of Infliximab and Adalimumab Are Associated With Mucosal Healing in Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2016; 14: 550-557.
14. Roblin X, Boschetti G, Duru G, Williet N, Deltedesco E, Phelip JM, Peyrin-Biroulet L, Nancey S, Flourie B, Paul S. Distinct Thresholds of Infliximab Trough Level Are Associated with Different Therapeutic Outcomes in Patients with Inflammatory Bowel Disease: A Prospective Observational Study. *Inflamm Bowel Dis* 2017; 23: 2048-2053.
15. D'Haens G, Vermeire S, Lambrecht G, Baert F, Bossuyt P, Pariente B, Buisson A, Bouhnik Y, Filippi J, Vander Woude J, Van Hootegem P, Moreau J, Louis E, Franchimont D, De Vos M, Mana F, Peyrin-Biroulet L, Brixi H, Allez M, Caenepeel P, Aubourg A, Oldenburg B, Pierik M, Gils A, Chevret S, Laharie D, GETAID. Increasing infliximab dose based on symptoms, biomarkers, and serum drug concentrations does not increase clinical, endoscopic, or corticosteroid-free remission in patients with active luminal Crohn's disease. *Gastroenterology* 2018; 154: 1343-1351.
16. The European Medicines Agency and the European Commission. Biosimilars in the EU, Information guide for healthcare professionals. 2017: 1-36.



Appendix



Nederlandse samenvatting

GERICHTE ANTI-TNF THERAPIE BIJ ERNSTIGE SARCOÏDOSE: RICHTING THERAPIE OP MAAT

Sarcoïdose

Sarcoïdose is een ziekte die gekenmerkt wordt door ontstekingen in verschillende delen van het lichaam. Deze ontstekingen kunnen overal in het lichaam voorkomen, maar komen het vaakst voor in de longen. Andere organen die vaak betrokken zijn bij de ziekte zijn de ogen, de huid en soms ook het hart en het zenuwstelsel. De symptomen van de ziekte kunnen zeer uiteenlopen en zijn afhankelijk van de organen waar de ontstekingen zich bevinden. Moeheid, koorts, benauwdheid, droge hoest, gewichtsverlies en algehele malaise behoren tot de meest voorkomende uitingen. De ziekte komt zowel bij mannen als bij vrouwen voor en ontwikkelt zich meestal in de leeftijd tussen de 20 en 40 jaar. Sarcoïdose is een zeldzame ziekte: in Nederland is de incidentie naar schatting 20 per 100.000 inwoners per jaar en de prevalentie wordt geschat op ongeveer 50 per 100.000 inwoners.

Het stellen van de diagnose sarcoïdose gebeurt meestal op basis van een combinatie van bevindingen: symptomen, een radiologisch beeld, een biopt met daarin granulomen (ophopingen van ontstekingscellen) en door het uitsluiten van andere oorzaken van granuloomvorming, zoals bepaalde infecties. In ongeveer de helft van de gevallen is er sprake van spontane verbetering en is behandeling met medicijnen niet nodig. Maar er is ook een groep patiënten met ernstigere ziekteverschijnselen. Bij deze patiënten kunnen de ontstekingen leiden tot orgaanschade en in sommige gevallen zelfs tot overlijden. Het is dus van belang dat deze patiënten wel behandeld worden. We weten niet precies hoe en waarom de ziekte ontstaat. Daarom is er geen genezende behandeling. De medicijnen die wel beschikbaar zijn kunnen de overreactie van het immuunsysteem onderdrukken, en daarmee de symptomen verminderen.

Behandeling van sarcoïdose met medicijnen

Een patiënt krijgt een behandeling met medicijnen indien de functie van een orgaan bedreigd wordt door sarcoïdose of als er sprake is van een sterke vermindering van de kwaliteit van leven. In eerste instantie wordt gestart met prednison, een corticosteroïd. Een van de nadelen van prednison is dat het bij langdurig gebruik veel bijwerkingen geeft, zoals gewichtstoename, suikerziekte en botontkalking. Als prednison onvoldoende effectief is of wanneer ernstige bijwerkingen optreden kan er overgestapt worden naar een tweedelijns middel. Het meest gebruikte tweedelijns middel is methotrexaat, maar ook azathioprine en leflunomide worden gebruikt.

Wanneer ook tweedelijns therapie onvoldoende effectief blijkt of ernstige bijwerkingen geeft, wordt overgestapt op derdelijns middelen. Deze derdelijns middelen zijn de anti-TNF *biologicals* infliximab en adalimumab. Deze biotechnologische medicijnen behoren tot de groep van monoklonale antilichamen. Infliximab en adalimumab zijn specifiek gericht tegen tumor necrose factor alfa (TNF). TNF is een boodschappereiwit en betrokken bij de ontstekingsreactie. Het wordt geproduceerd door ontstekingscellen in twee vormen: als membraangebonden TNF,

waarbij het vastzit aan het oppervlak van de cel, of als vrij TNF. TNF bindt aan de TNF-receptor op andere ontstekingscellen. Het stimuleert deze cellen om zich te vermenigvuldigen en andere boodschappereiwitten te produceren die de ontstekingsreactie verder versterken. TNF speelt dus een zeer belangrijke rol in het creëren en op gang houden van de ontstekingsreactie bij sarcoïdose. Infliximab en adalimumab binden aan TNF en voorkomen hiermee dat TNF aan de TNF-receptor kan binden. Hierdoor kan TNF zijn signaal niet meer doorgeven. Op deze manier remmen infliximab en adalimumab de ontstekingsreactie.

Het meten van de respons

Om te bepalen of een patiënt met sarcoïdose goed reageert op bovengenoemde medicijnen kunnen verschillende metingen gedaan worden.

Afhankelijk van welke organen betrokken zijn bij de ziekte wordt gemeten hoe goed de betrokken organen functioneren. Omdat bij meer dan 90% van de patiënten de longen betrokken zijn, wordt vaak de longfunctie gemeten.

Daarnaast wordt gekeken hoe actief de ontsteking is. Dit kan op meerdere manieren, bijvoorbeeld in het bloed en met behulp van een scan. In het bloed kunnen zogeheten biomarkers gemeten worden. Hoe hoger deze biomarkers, hoe hoger de ontstekingsactiviteit. De meest gebruikte biomarkers voor ziekteactiviteit bij sarcoïdose zijn inflammatoire biomarkers 'angiotensine-converting enzyme' (ACE) en 'soluble interleukin-2 receptor' (sIL-2R). Een andere methode is gebaseerd op het inspuiten van radioactief suiker waarna een scan de ontsteking in beeld kan brengen. Het betreft hier de zogenaamde ^{18}F-FDG PET scan. Met deze methode kan naast de hoogte van de ziekteactiviteit ook in beeld worden gebracht waar de ziekteactiviteit in het lichaam zich bevindt.

Tot slot wordt gekeken naar de kwaliteit van leven. De kwaliteit van leven kan geëvalueerd worden met behulp van vragenlijsten. Uit eerder onderzoek is gebleken dat sarcoïdosepatiënten vooral op de gebieden fysiek functioneren en moeheid een verminderde kwaliteit van leven ervaren in vergelijking met mensen die geen sarcoïdose hebben. In medicijnenonderzoeken bij sarcoïdose is de invloed op de kwaliteit van leven tot nu toe weinig onderzocht.

Het doel van het onderzoek

Omdat sarcoïdose een zeldzame ziekte is en slechts 10% van de sarcoïdosepatiënten in aanmerking komt voor behandeling met anti-TNF middelen is er maar weinig bekend over het effect van de derdelijns *biologicals* bij ernstige sarcoïdose. Ook zijn er nog weinig gegevens beschikbaar over het optimale doseerregime en welke patiënten het meest baat hebben bij behandeling met deze middelen. Het doel van het onderzoek was om het effect van de anti-TNF middelen infliximab en adalimumab bij patiënten met ernstige sarcoïdose te onderzoeken. Daarnaast is onderzoek gedaan naar factoren die mogelijk van invloed zijn op de mate van reactie van een patiënt op de behandeling. Factoren die zijn onderzocht zijn genetische variaties, inflammatoire biomarkers in het bloed, de ^{18}F-FDG PET scan, de concentratie van het medicijn in het bloed en

de blootstelling van het lichaam aan deze anti-TNF middelen. Dit alles is gedaan om na te gaan of deze factoren uiteindelijk zouden kunnen helpen de behandeling preciezer af te stemmen op de individuele patiënt.

Hoofdstuk 1 is een inleidend hoofdstuk. **Hoofdstuk 2** geeft een uitgebreid overzicht van de huidige kennis over anti-TNF middelen bij sarcoïdose. Bij sarcoïdose worden twee typen anti-TNF middelen gebruikt.

Allereerst zijn er de niet-specifieke immuun-modulerende geneesmiddelen die de aanmaak van TNF remmen. Voorbeelden zijn thalidomide, pentoxifylline en apremilast. In de klinische praktijk worden deze middelen echter niet vaak voorgeschreven omdat ze beperkt zijn onderzocht bij sarcoïdose. Daarnaast laten de gegevens die wel beschikbaar zijn een ongunstige balans tussen effectiviteit en veiligheid zien. De gepubliceerde studies beschrijven wisselende resultaten over de effectiviteit. Bovendien komen bijwerkingen, zoals slaperigheid en misselijkheid, bij veel patiënten voor.

De tweede groep is die van de anti-TNF *biologicals*. Deze middelen zijn specifiek gericht tegen TNF. Voorbeelden zijn infliximab, adalimumab en etanercept. Bij sarcoïdose worden ze gegeven als eerste- en tweedelijns middelen onvoldoende werkzaam zijn gebleken. Studies met infliximab en adalimumab laten zien dat deze middelen effectief kunnen zijn bij patiënten met ernstige sarcoïdose. Etanercept heeft echter onvoldoende effectiviteit laten zien bij sarcoïdose. Dit verschil wordt mogelijk veroorzaakt doordat de binding van etanercept aan de membraangebonden vorm van TNF minder sterk is dan de binding van infliximab en adalimumab aan deze vorm van TNF. Ook hebben experimenten met cellen laten zien dat etanercept niet in staat is om celdood van ontstekingscellen te veroorzaken, terwijl infliximab en adalimumab daar wel toe in staat waren.

Naast de gunstige effecten van anti-TNF *biologicals*, zijn er ook diverse bijwerkingen gemeld, zoals infecties en allergische reacties. Symptomen van allergische reacties zijn onder andere koorts en daling van de bloeddruk. Deze allergische reacties kunnen worden veroorzaakt doordat het lichaam een afweerreactie ontwikkelt tegen de anti-TNF *biological*. Hierbij worden antilichamen tegen het medicijn geproduceerd. Deze antilichamen binden aan het medicijn waardoor het niet meer goed aan TNF kan binden en het medicijn zijn werking niet meer kan uitoefenen.

Omdat er maar weinig studies met veel patiënten zijn gedaan, zijn de huidige richtlijnen voor de behandeling van ernstige sarcoïdose met anti-TNF *biologicals* voornamelijk gebaseerd op ervaringen van sarcoïdose-experts. Niet alle patiënten met ernstige sarcoïdose hebben baat bij behandeling met anti-TNF *biologicals*. Daarom is er een sterke behoefte aan meer gegevens over de effectiviteit van anti-TNF *biologicals* bij ernstige sarcoïdose en naar factoren die de werking van deze medicijnen beïnvloeden.

In **hoofdstuk 3** worden de resultaten beschreven van een prospectieve, observationele studie naar het effect van infliximab bij ernstige sarcoïdose. Er werden 56 patiënten geïncludeerd die

eerder onvoldoende reageerden op eerste- en tweedelijns middelen. Patiënten kregen via een infuus een dosis infliximab van 5 mg/kg bij de start van de behandeling, daarna na 2 weken en vervolgens elke 4 weken. Op basis van de inflammatoire biomarkers sIL-2R en ACE en ziekteactiviteit op de ^{18}F-FDG PET scan hadden de meeste patiënten een hoge ziekteactiviteit bij de start van de behandeling met infliximab. Na 6 maanden werd het effect van infliximab op orgaanfunctie, ontstekingsactiviteit en kwaliteit van leven geëvalueerd. De longfunctieparameter 'forced vital capacity' (FVC) verbeterde gemiddeld met 6,6% van voorspeld (p=0,0007). De bloedwaarden van de inflammatoire biomarkers sIL-2R en ACE en ziekteactiviteit op de ^{18}F-FDG PET scan daalden sterk, wat wijst op een afname van de ontstekingsactiviteit van sarcoïdose. Er werd een verband gevonden tussen de ziekteactiviteit gemeten in de longen met de ^{18}F-FDG PET scan voor de start van de behandeling en de verbetering van de longfunctieparameter FVC na 6 maanden behandeling: hoe hoger de ziekteactiviteit voor de start van de behandeling met infliximab, hoe groter de verbetering in longfunctieparameter FVC na behandeling met infliximab (R=0,62; p=0,0004). Ook de kwaliteit van leven verbeterde: op de visuele analoge schaal (een schaal van 0 - 100) scoorden patiënten 14,6 punten beter (p<0,0001) na 6 maanden behandeling met infliximab. Bij 79% van de patiënten werd een goed effect gevonden op minimaal twee van de drie geëvalueerde gebieden (orgaanfunctie, ontstekingsactiviteit, kwaliteit van leven). De gemiddelde infliximabconcentratie in het bloed vlak voor de volgende dosis was 18 µg/mL. Dit is hoger dan bij andere ziekten die ook behandeld worden met infliximab. De infliximabconcentratie in het bloed was niet gerelateerd aan het therapeutisch effect van infliximab.

Deze studie laat zien dat infliximab een effectief middel is bij patiënten met ernstige sarcoïdose en dat het meten van de ziekteactiviteit met de ^{18}F-FDG PET scan mogelijk van toegevoegde waarde kan zijn bij het selecteren van patiënten voor deze behandeling.

Uit eerder onderzoek is bekend dat sarcoïdosepatiënten die binnen een jaar stoppen met de infliximabbehandeling, vaak terugkeer van de ziekteverschijnselen laten zien. Patiënten worden daarom meestal gedurende lange tijd behandeld. Er is echter maar weinig bekend over het lange termijn effect van infliximab bij sarcoïdose: blijft het werkzaam, neemt de werkzaamheid verder toe of neemt het af? Daarom wordt in **hoofdstuk 4** het effect van infliximab bij langdurig gebruik onderzocht. Er werden 37 patiënten geïncludeerd die minimaal een jaar met infliximab waren behandeld. Van deze 37 patiënten gingen 26 patiënten door met de infliximab behandeling gedurende minimaal 2 jaar. Tijdens de studie werd de orgaanfunctie iedere 6 maanden gemeten. De ontstekingsactiviteit werd met behulp van bloedwaarden van de inflammatoire biomarkers sIL-2R en ACE iedere drie maanden bepaald. De verbetering in orgaanfunctie die na de eerste 6 maanden van behandeling met infliximab werd gezien bleef in de periode daarna stabiel. De bloedwaarden van inflammatoire biomarkers waren na de eerste 3 maanden behandeling met infliximab sterk gedaald en ook deze bleven daarna stabiel. Dit betekent dus dat het behaalde resultaat van infliximabtherapie bij sarcoïdose gehandhaafd blijft bij het voortzetten van die therapie: het effect neemt niet verder toe en neemt ook niet af.

Adalimumab is ook een *biological* die specifiek bindt aan TNF. Er zijn echter veel minder gegevens beschikbaar over de effectiviteit en veiligheid van adalimumab bij sarcoïdose dan over infliximab. Daarom wordt in de praktijk de voorkeur gegeven aan infliximab bij behandeling van sarcoïdose. Echter, ongeveer 10% van de patiënten met sarcoïdose die behandeld worden met infliximab krijgt een afweerreactie tegen het middel en ontwikkelt antilichamen tegen infliximab. Hierdoor is infliximab niet meer werkzaam en kan de ziekte weer actief worden. Daarnaast hebben sommige patiënten last van allergische reacties tijdens of na het toedienen van infliximab. Voor deze patiënten is dus een alternatief nodig. Meestal stappen deze patiënten over naar behandeling met adalimumab. De antistoffen tegen infliximab reageren namelijk niet op adalimumab. Ook patiënten die last hebben van ernstige bijwerkingen van infliximab die niet of minder vaak voorkomen bij adalimumab, kunnen overstappen. Over de effectiviteit en veiligheid van adalimumab in deze groep patiënten zijn echter geen gegevens beschikbaar. In **hoofdstuk 5** wordt onderzocht of adalimumab stabilisatie of verbetering van sarcoïdose kan bewerkstelligen bij patiënten die aanvankelijk goed reageerden op infliximab maar die vanwege antilichamen tegen infliximab of vanwege bijwerkingen infliximab niet meer kunnen verdragen. Van 142 patiënten die behandeld werden met infliximab, stapten 18 patiënten (13%) over naar adalimumab. Na een jaar behandeling met adalimumab verbeterde de orgaanfunctie in 7 patiënten (39%), bleef stabiel in 6 patiënten (33%) en verslechterde in 5 patiënten (28%). De ziekteactiviteit, gemeten met behulp van de inflammatoire biomarker sIL-2R, nam bij geen van de patiënten toe tijdens de behandeling met adalimumab. De meest voorkomende bijwerking was een infectie (n=10). Deze studie laat zien dat bij 72% van de patiënten de ziekte stabiel bleef bij de overstap naar adalimumab bij patiënten die infliximab niet langer kunnen verdragen. Adalimumab is dus een geschikt alternatief voor infliximab bij patiënten die intolerant zijn geworden voor infliximab.

Helaas hebben niet alle patiënten baat bij infliximabtherapie: in hoofdstuk 3 zagen we dat 21% van de patiënten slechts gedeeltelijk of niet reageert op behandeling met infliximab. Bij voorkeur starten we de behandeling met infliximab alleen bij patiënten die goed zullen gaan reageren op dit middel. Infliximab kan ernstige bijwerkingen geven, zoals tuberculose, ernstige andere infecties en allergische reacties. Daarnaast is het een duur geneesmiddel. Het is dus van belang om te zoeken naar factoren die kunnen voorspellen of een patiënt gunstig zal reageren op infliximab. Uit onderzoek naar het effect van infliximab bij andere auto-immuunziekten weten we dat variaties in het DNA (genetische variaties) van invloed kunnen zijn op het effect van infliximab. In **hoofdstuk 6** wordt onderzocht of er een verband is tussen variaties in genen en het effect van infliximab bij sarcoïdose. Hiervoor zijn de genen die coderen voor TNF, de TNF-receptoren 1 en 2 (*TNFRSF1A* en *TNFRSF1B*), Fcγ-receptoren en HLA onderzocht. In deze studie werd bij 106 patiënten gevonden dat infliximab bij patiënten die drager waren van het *TNFRSF1A* rs1800693 G-allel (patiënten met het GG- en GA-genotype) een minder grote invloed had op de longfunctieparameter DLCOc (een maat voor de mate van gaswisseling in de long) en inflammatoire biomarkers in het bloed, dan bij patiënten die geen drager waren van het G-allel (patiënten met

het AA-genotype). Opvallend was dat patiënten met het *TNFRSF1A* rs1800693 GG- en GA-genotype ook een betere DLCOc en minder ziekteactiviteit op basis van inflammatoire biomarkers hadden voorafgaand aan de start van de infliximabbehandeling, dan patiënten met het AA-genotype. Het zou dus kunnen dat de minder gunstige uitkomst bij patiënten met het *TNFRSF1A* rs1800693 GG- of GA-genotype samenhangt met de waarneming dat de sarcoïdose minder ernstig is en de ziekteactiviteit lager is bij deze patiënten, dan bij patiënten met het AA-genotype. Bij dragers van het *TNFRSF1B* T196G T-allel (TT- en TG-genotype) daalde de bloedwaarde van de inflammatoire biomarker ACE, terwijl bij patiënten met het GG-genotype ACE gemiddeld toenam. De afwezigheid van de genetische variatie HLA-DRB*0301 in het HLA-gebied liet een verband zien met een grotere daling van de sIL-2R concentraties vergeleken met HLA-DRB*0301-positieve patiënten.

Er werd geen verband gevonden tussen de genen die coderen voor TNF en Fcγ-receptoren en het effect van infliximab.

Concluderend, er werd in deze studie een verband gevonden tussen genetische variaties in *TNFRSF1A*, *TNFRSF1B* en HLA en de verandering in de longfunctieparameter DLCOc en inflammatoire parameters na behandeling met infliximab bij sarcoïdose. Verdere studies naar deze genetische variaties zijn nodig om deze bevindingen te bevestigen en de implicaties voor het kiezen van een optimale therapie vast te stellen.

In **hoofdstuk 7** werd een populatie-farmacokinetisch model voor infliximab bij sarcoïdose ontworpen. Dit model beschrijft het verloop van de infliximabconcentratie in het bloed over de tijd. Bij 68 patiënten werd meerdere malen bloed afgenomen om de infliximabconcentratie te bepalen: topconcentraties (bloedafname 1 uur na het einde van de infliximabinfusie), twee weken na toediening van infliximab en de dalconcentraties (vlak voor toediening van de volgende gift infliximab) werden gebruikt om een populatie-farmacokinetisch model met twee compartimenten te ontwikkelen. Hiervoor werd het computerprogramma NONMEM gebruikt. De geschatte waarden van de populatie farmacokinetische parameters (typische waarde (relatieve standaardafwijking)) waren als volgt: systemische klaring (CL) 0,276 L/dag (3,2%), volume van het centrale compartiment (V1) 3,16 L (1,6%), intercompartimentele klaring (Q) 0,177 L/dag (21%) en volume van het perifere compartiment (V2) 1,49 L (11%). Deze waarden zijn vergelijkbaar met eerder beschreven populatie-farmacokinetische modellen voor infliximab bij andere auto-immuunaandoeningen. In een covariatenanalyse werd gevonden dat lichaamsoppervlakte, lichaamsgewicht, serum albumine en aanwezigheid van antistoffen tegen infliximab de populatie-farmacokinetische parameters (en daarmee het concentratieverloop) van infliximab beïnvloeden. Er werd geen verband gevonden tussen de infliximabconcentratie in het bloed of de mate van blootstelling aan infliximab en het therapeutisch effect. Een mogelijke verklaring hiervoor is dat de infliximabconcentratie in het bloed hoger is dan nodig. Nieuwe studies zijn van belang om vast te stellen welke infliximabconcentratie in het bloed nagestreefd dient te worden bij de behandeling van sarcoïdose met infliximab.

TOEKOMSTPERSPECTIEVEN EN CONCLUSIES

In dit proefschrift zijn meerdere aspecten rondom de behandeling van sarcoïdose met anti-TNF middelen onderzocht met als doel om de behandelrichtlijnen voor deze patiëntengroep beter te onderbouwen. Daarnaast hebben we een aantal nieuwe factoren geïdentificeerd die in de toekomst mogelijk kunnen helpen bij het verder optimaliseren en het op maat instellen van de medicamenteuze behandeling van sarcoïdose.

Op basis van onze bevindingen kunnen een aantal aanbevelingen voor de dagelijkse praktijk gedaan worden:

Bij patiënten met ernstige sarcoïdose is er een verband tussen ziekteactiviteit bij de start van de behandeling met infliximab en de mate van verbetering van de longfunctie na de behandeling. Criteria voor het selecteren van sarcoïdosepatiënten voor behandeling met infliximab omvatten dus idealiter zowel de ernst van de ziekte als de inflammatoire activiteit.

Daarnaast is gevonden dat het effect van infliximab na 6 maanden behandeling behouden blijft bij het continueren van de behandeling. Het advies is dan ook om, indien een patiënt een goede respons laat zien, de behandeling met infliximab te continueren (indien het risico op reactivatie als te groot wordt ingeschat).

Voor patiënten die aanvankelijk goed reageren op infliximab maar die intolerant worden voor het middel, is adalimumab een goed alternatief.

Er zijn ook een aantal bevindingen die aanleiding zijn voor vervolgonderzoek:

In het onderzoek hebben we geen relatie gevonden tussen de concentratie infliximab in het bloed of de blootstelling aan infliximab en het therapeutisch effect. Bij een andere auto-immuunaandoening, inflammatoire darmziekten, is wel een verband gevonden tussen de infliximabconcentratie in het bloed en het waargenomen effect. Een mogelijke oorzaak voor dit verschil is dat bij sarcoïdose met het huidige doseerregime de infliximabconcentratie in het bloed hoger is dan nodig. Wat de optimale infliximabconcentratie in het bloed is bij sarcoïdose, is nog niet bekend. Hiernaar is verder onderzoek wenselijk.

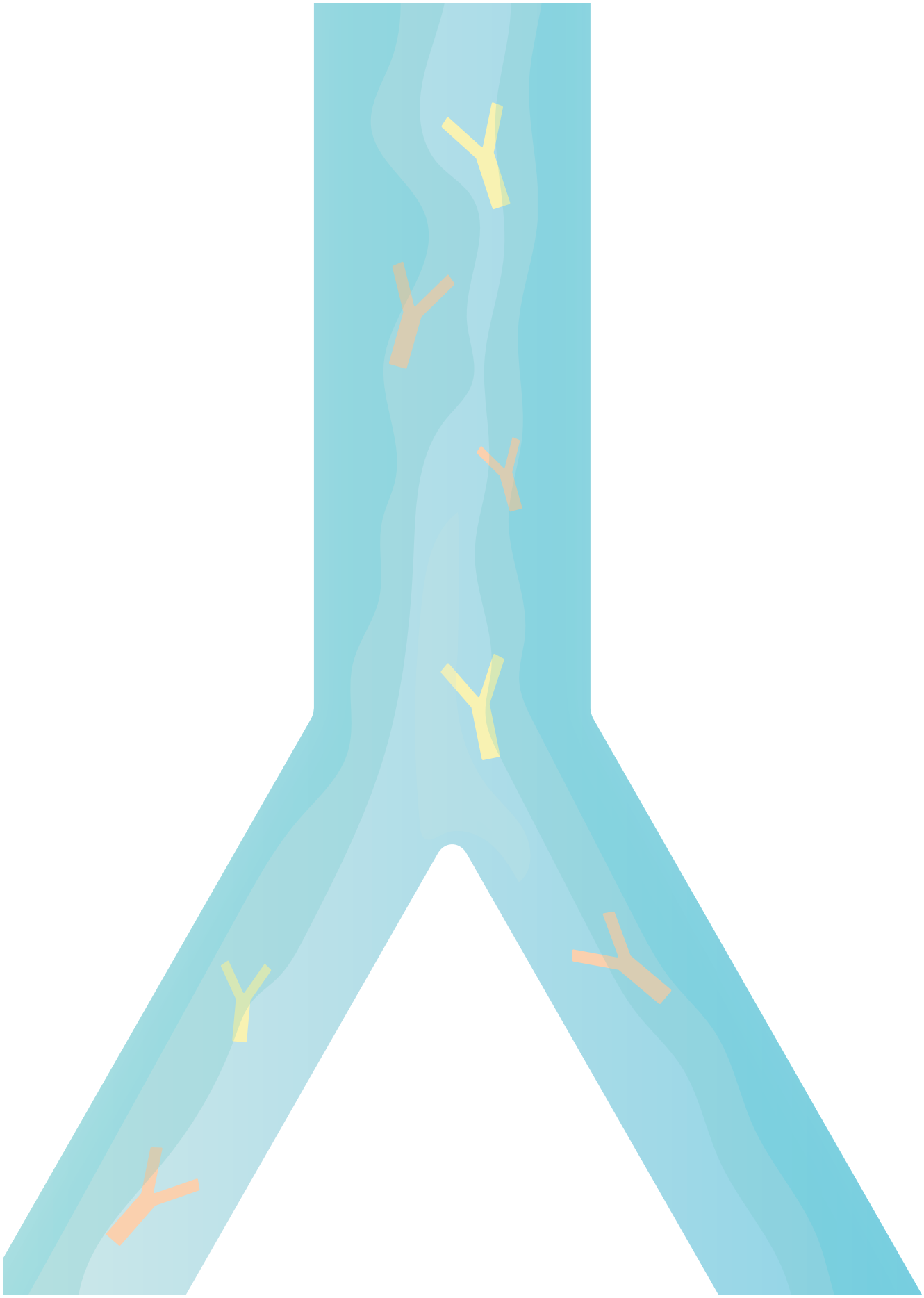
Doel van het onderzoek was om factoren die het effect van anti-TNF middelen bij sarcoïdose beïnvloeden te identificeren om zo patiënten te selecteren die het meest baat hebben bij de behandeling. Er is een verband gevonden tussen een hogere ziekteactiviteit bij de start van infliximabtherapie en de verbetering van de longfunctie. Daarnaast is het effect van infliximab groter bij patiënten met bepaalde genetische variaties dan bij patiënten zonder deze variaties. Dit zijn nieuwe inzichten die kunnen helpen onderscheid te maken tussen patiënten die waarschijnlijk wel en patiënten die waarschijnlijk niet zullen reageren op de behandeling met infliximab. Het is echter niet aannemelijk dat een enkele variabele dit onderscheid zal kunnen maken; waarschijnlijker is dat een combinatie van meerdere variabelen zoals biomarkers, klinische parameters en genotypes zal kunnen helpen bij het voorspellen van het therapeutisch voordeel van het inzetten van infliximab bij sarcoïdose.

Sarcoïdose is een heterogene ziekte die op veel verschillende manieren tot uiting kan komen. Dit bemoeilijkt het vaststellen van een universele manier om het effect te beschrijven. In dit proefschrift zijn meerdere uitkomstmaten gebruikt om het effect van infliximab- en adalimumabtherapie vast te stellen. Een gouden standaard voor het meten van het effect van medicijnen bij sarcoïdose zou van grote waarde zijn. Het zou helpen om uitkomsten van klinische studies beter met elkaar te kunnen vergelijken en om de bevindingen van klinische studies op een juiste wijze te implementeren in de praktijk.

Sinds kort zijn van infliximab naast het originele product (Remicade®) zogenaamde 'biosimilars' beschikbaar. Biosimilars zijn generieke versies van complexe medicijnen, zoals infliximab. Ze zijn gelijkwaardig aan een bestaand biologisch geneesmiddel. Over het algemeen zijn biosimilars goedkoper dan het originele product. Geneesmiddelautoriteiten stellen hoge eisen aan de kwaliteit van biosimilars: om toegelaten te worden, worden ze uitgebreid getest in het laboratorium en ook in patiënten die lijden aan ziekten waarvoor het originele product geregistreerd is. Omdat Remicade® niet geregistreerd is voor gebruik bij sarcoïdose, zullen de biosimilars in het traject voor registratie niet bij sarcoïdosepatiënten getest worden. Het populatie-farmacokinetiek model dat beschreven is in dit proefschrift, kan bruikbaar zijn in toekomstige studies om vast te stellen of een biosimilar zich op dezelfde manier gedraagt in het lichaam als het oorspronkelijke product.

Omdat sarcoïdose een zeldzame ziekte is en slechts 10% van de sarcoïdosepatiënten in aanmerking komt voor behandeling met anti-TNF *biologicals*, is het onwaarschijnlijk dat medicijnen ontwikkeld worden speciaal voor patiënten met ernstige sarcoïdose. Daarom is het van belang om de ontwikkelingen van de behandeling van andere auto-immuunziekten met een vergelijkbare (waarschijnlijke) oorzaak nauwgezet te volgen. Medicijnen die op basis van hun werkingsmechanisme waarschijnlijk ook een effect zullen hebben bij sarcoïdosepatiënten zouden onderzocht kunnen worden in klinische studies. Hopelijk leidt dit in de toekomst tot meer behandelopties voor patiënten met ernstige sarcoïdose.

Het onderzoek beschreven in dit proefschrift vormt een eerste stap in de richting van therapie op maat. Hopelijk draagt het daarmee bij aan verbetering van de zorg voor patiënten met ernstige sarcoïdose en inspireert het onderzoekers in de zoektocht naar het beter begrijpen en beter kunnen behandelen van deze nog zo raadselachtige ziekte.



Affiliations of the authors

AFFILIATIONS OF THE AUTHORS

(in order of appearance in the manuscript)

Heleen A Crommelin
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Department of Clinical Pharmacology, Division of Laboratory Medicine & Pharmacy, University Medical Center Utrecht, Utrecht, The Netherlands

Jan C Grutters
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands

Vera HM Deneer
Department of Clinical Pharmacology, Division of Laboratory Medicine & Pharmacy, University Medical Center Utrecht, Utrecht, The Netherlands

Coline HM van Moorsel
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands

Adriane DM Vorselaars
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands

Ingrid HE Korenromp
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands

Bob Meek
Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands

Anke ME Claessen
Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands

Ruth GM Keijsers
Department of Nuclear Medicine, St Antonius Hospital, Nieuwegein, The Netherlands

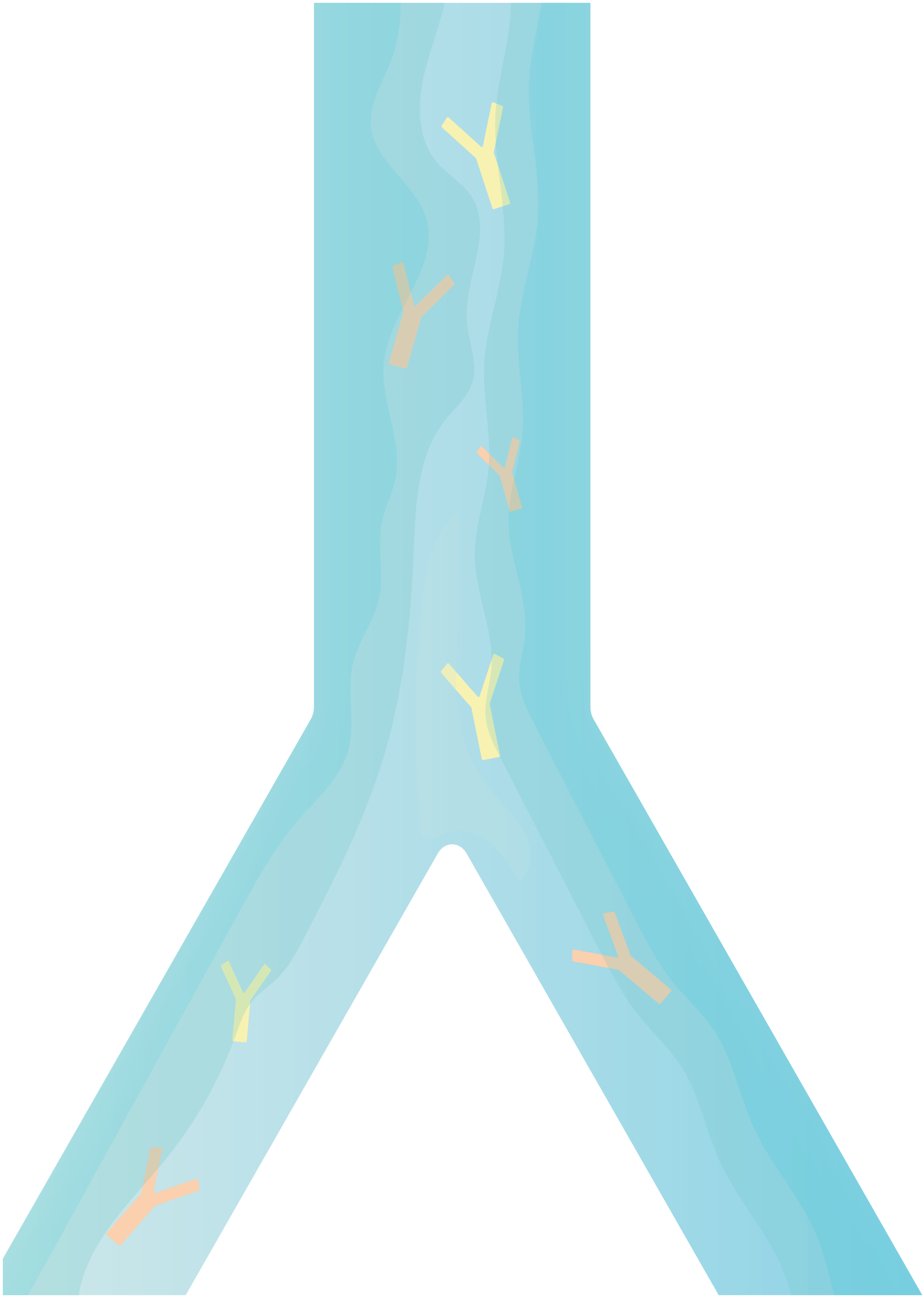
Leone M van der Burg
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Science Department, University College Roosevelt, Middelburg, The Netherlands

Marjolein Drent
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands

Ger T Rijkers
Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands
Science Department, University College Roosevelt, Middelburg, The Netherlands

Joanne J van der Vis
Interstitial Lung Diseases Center of Excellence, Department of Pulmonolgy, St Antonius Hospital, Nieuwegein, The Netherlands
Interstitial Lung Diseases Center of Excellence, Department of Clinical Chemistry, St Antonius Hospital, Nieuwegein, The Netherlands

Johannes H Proost
Department of Pharmacokinetics, Toxicology and Targeting, University of Groningen, Groningen, The Netherlands



Dankwoord / Acknowledgments

DANKWOORD / ACKNOWLEDGMENTS

Dat je deze pagina hebt opengeslagen en dit dankwoord leest, betekent over het algemeen niet dat je alle voorgaande pagina's van dit proefschrift (al) hebt gelezen. Maar dat geeft niet: zonder de steun en begeleiding van velen zou het me niet gelukt zijn dit proefschrift af te ronden. Het is dus eigenlijk logisch om bij het dankwoord te beginnen.

Ik kijk terug op een leerzame, leuke en soms stressvolle periode. Alle ingrediënten om veel over wetenschap, onderzoek en over mezelf te leren, waren er. Iedereen die hierbij betrokken is geweest wil ik daarom hartelijk bedanken, in het bijzonder de volgende mensen.

Allereerst wil ik mijn co-promotor dr. V.H.M. Deneer bedanken. Beste Vera, de afgelopen jaren heb je me geweldig begeleid, eerst in het St Antonius Ziekenhuis en daarna in het UMC Utrecht. De kansen die je me hebt geboden als onderzoeker en om als apotheker ervaring op te doen op het lab, waardeer ik enorm. Je hebt me geleerd een probleem op een gestructureerde manier te analyseren en aan te pakken. Ik heb veel respect voor hoe je naast al je drukke werkzaamheden op de een of andere manier tijd maakt om me verder te helpen: ik kon en kan altijd bij je terecht. Ik had me geen betere co-promotor kunnen wensen.

Graag wil ik mijn promotor, prof. dr. J.C. Grutters, danken voor de uitstekende begeleiding. Beste Jan, je bent een van de meest inspirerende mensen die ik ken. Ik heb respect voor hoe je veel verschillende vakgebieden samenbrengt in het onderzoek en ook in de dagelijkse klinische praktijk: van fundamentele studies over telomeerlengte tot het bestuderen van het effect van medicijnen op de kwaliteit van leven in patiëntstudies. En dat is terug te zien in de grote variatie aan vakgebieden en mensen in je onderzoeksgroep.

Ook wil ik mijn co-promotor dr. C.H.M. van Moorsel bedanken. Beste Coline, de manier waarop je me hebt begeleid heeft ertoe bijgedragen dat ik meer uit mezelf en uit het onderzoek heb kunnen halen, dan ik had gedacht. Ik bewonder je vermogen om in te spelen op de persoonlijke behoefte van iedere onderzoeker. Van onze gesprekken op de late vrijdagmiddag over hoe de onderzoekswereld in elkaar zit/zou moeten zitten heb ik genoten. Je kritische blik, en op de goede momenten een schouderklopje, waardeer ik zeer.

Vera, Jan en Coline, jullie samenwerking om mij verder te helpen wil ik hier zeker noemen. Dat jullie voornaamste doel was om samen mij verder te helpen met mijn (wetenschappelijke) carrière, leek me aanvankelijk te mooi om waar te zijn. Maar ik zie nu dat het dé manier is om mensen het meest uit zichzelf te kunnen laten halen. Dank voor dit goede voorbeeld.

&

Graag wil ik alle co-auteurs van de diverse hoofdstukken bedanken voor hun enthousiasme, kritische blik en goede suggesties.

Renske, we lijken niet op elkaar in onze manier van werken. Ondanks dat, en misschien juist wel daarom, is onze samenwerking in de loop van de jaren alleen maar beter geworden. Jij de grote lijnen, ik de details. Bedankt voor je hulp zowel tijdens als nadat je jouw proefschrift had afgerond. Annette, dank voor je hulp bij het genotyperen. Maar bovenal dank dat ik altijd bij je terecht kon voor een luisterend oor, zowel op de onderzoekskamer als tijdens onze fietsritjes.
Danielle, bedankt voor alle analyses en de prachtige overzichten. Dan denk ik meteen aan het grote overzichtsdocument met de data van alle afnames, inclusief kleurtjes. En dank voor je interesse, zowel op werkgebied als persoonlijk vlak, ook nadat je elders was gaan werken.

De analisten van het FarmaToxLab van het St Antonius Ziekenhuis wil ik bedanken: Lilian en Kees, veel dank voor het bepalen van de meer dan 1.000(!) infliximabspiegels. Aan Sylvia, Remko, Richard, Tamimount en Peter dank voor het verwerken van alle monsters. En dank voor de gezelligheid op en rond het FarmaToxLab.

De medewerkers van de specialistische poli ILD en infuus-afdeling wil ik bedanken voor de zorgen rondom inclusie en behandeling van patiënten. En de medewerkers van de laboratoria van de MMI en KCL dank ik voor het verwerken van de monsters.

ILD-verpleegkundigen Elma, Miranda, Annemiek, Mirjam, Sebastiaan, Evelien en Lian, jullie hebben me geleerd altijd een stap vooruit te denken. Bedankt voor jullie enthousiasme en interesse in het onderzoek.

Alle leden van de onderzoeksgroep Reinier, Annemarie, Renske, Bekir, Liesbeth, Annette, Karin (dank voor je immer kritische vragen), Marjolijn, Anouk, Thijs, Ivo, Dymph, Aernoud, Inge, Els, Anna, Nynke, Sofia, Hanneke, Claudia, Kim, Yvonne, Helmi, Marjolein, Marleen, Ingrid, Annelies en de rest van de onderzoeksgroep, bedankt voor jullie hulp en gezelligheid op de onderzoekskamer en tijdens congressen, voor de ijsjes, de borrels, de etentjes en de vele taart. Dank voor het samen genieten van de successen en het oppeppen van elkaar bij tegenvallers.

Studenten Franka en Leone dank voor jullie inzet, enthousiasme en bijdrage aan dit onderzoek.

(Ziekenhuis)apothekers van het St Antonius Ziekenhuis, bedankt voor de leerzame tijd, gezelligheid, skivakanties en interesse in mijn onderzoek.

Tijdens het laatste stuk van mijn promotietraject was ik werkzaam in het UMC Utrecht. Graag dank ik de (ziekenhuis)apothekers, en in het bijzonder de opleiders Karin Rademaker en Ingeborg

Wilting, voor hun hulp en het meedenken in de afgelopen periode om dit promotieonderzoek tot een goed einde te brengen. Dank aan mede-AIOS en AIOS-kamer-(aka huiskamer-)genoten Heshu, Bastiaan, Anouk, Koen, Bart, Laura, Laurent, Raween, Niels: dank voor jullie steun en aanmoedigingen, vooral tijdens de laatste loodjes (want die wegen toch echt wel zwaar).

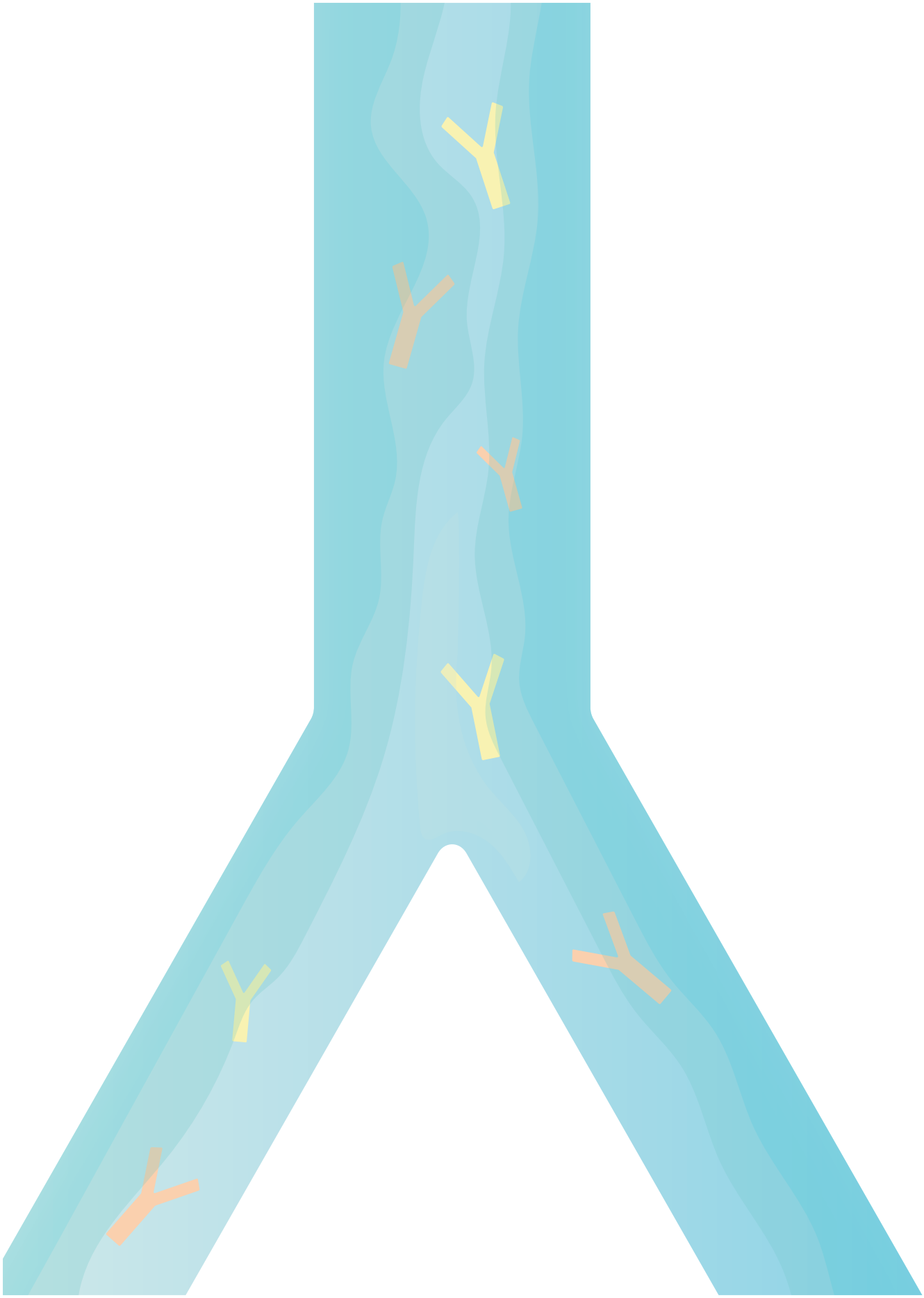
Lisanne en Hanneke, ik ben blij dat jullie mijn paranimfen willen zijn en dat jullie tijdens mijn verdediging aan mijn zijde willen staan. Lisanne, dank voor je oprechte interesse in mijn werkzaamheden. We leerden elkaar kennen op het hockeyveld, maar hebben sindsdien veel andere avonturen beleefd. Met jouw organisatievermogen weet ik dat ik onbezorgd kan genieten op 29 mei.
Hanneke, in onze studietijd leidde jij me naar het roeien en daarna naar het wielrennen. Met je nuchterheid loods je me ongetwijfeld in alle rust door de dag van mijn verdediging heen.

Lieve vrienden, jaarclub Dulcamara, Festina Lente, EJD'08 en andere roeivrienden en –vriendinnen, de hockey-vrouwen van Zwaluwen en Kampong en Daan, studiegenootjes en andere vrienden, dank voor jullie interesse, begrip, vriendschap en support de afgelopen jaren. En bovenal voor de gezonde portie relativering op z'n tijd.

Lieve familie, dank voor jullie grote interesse in mijn onderzoek en het begrip als ik laat aansloot op 1e kerstdag omdat er zonodig een artikel afgeschreven moest worden.
Olivera, je bent als een zus voor me. Dank voor je support de afgelopen jaren.

Lieve mama en papa, dank voor jullie onvoorwaardelijke steun, grote support, positiviteit en de pastamaaltijden, waarna mijn hersenen altijd weer meer zin kregen om er vol tegen aan te gaan. Dank dat jullie altijd voor me klaarstaan.

&



List op publications

LIST OF PUBLICATIONS

Schimmelpennink MC, Vorselaars ADM, van Beek FT, **Crommelin HA**, Deneer VHM, Keijsers RGM, Veltkamp M. Efficacy and safety of infliximab biosimilar Inflectra® **in severe** sarcoidosis. *Respir Med* 2018; in press.

Crommelin HA, van der Burg LM, Vorselaars ADM, Drent M, van Moorsel CHM, Rijkers GT, Deneer VHM, Grutters JC. Efficacy of adalimumab in sarcoidosis patients who developed intolerance to infliximab. *Respir Med* 2016, 115: 72-77.

Hijdra D, Vorselaars ADM, **Crommelin HA**, van Moorsel CHM, Meek B, Claessen AM, Rijkers GT, Grutters JC. Can intermediate monocytes predict response to infliximab therapy in sarcoidosis? *Eur Respir J* 2016, 48(4): 1242-1245.

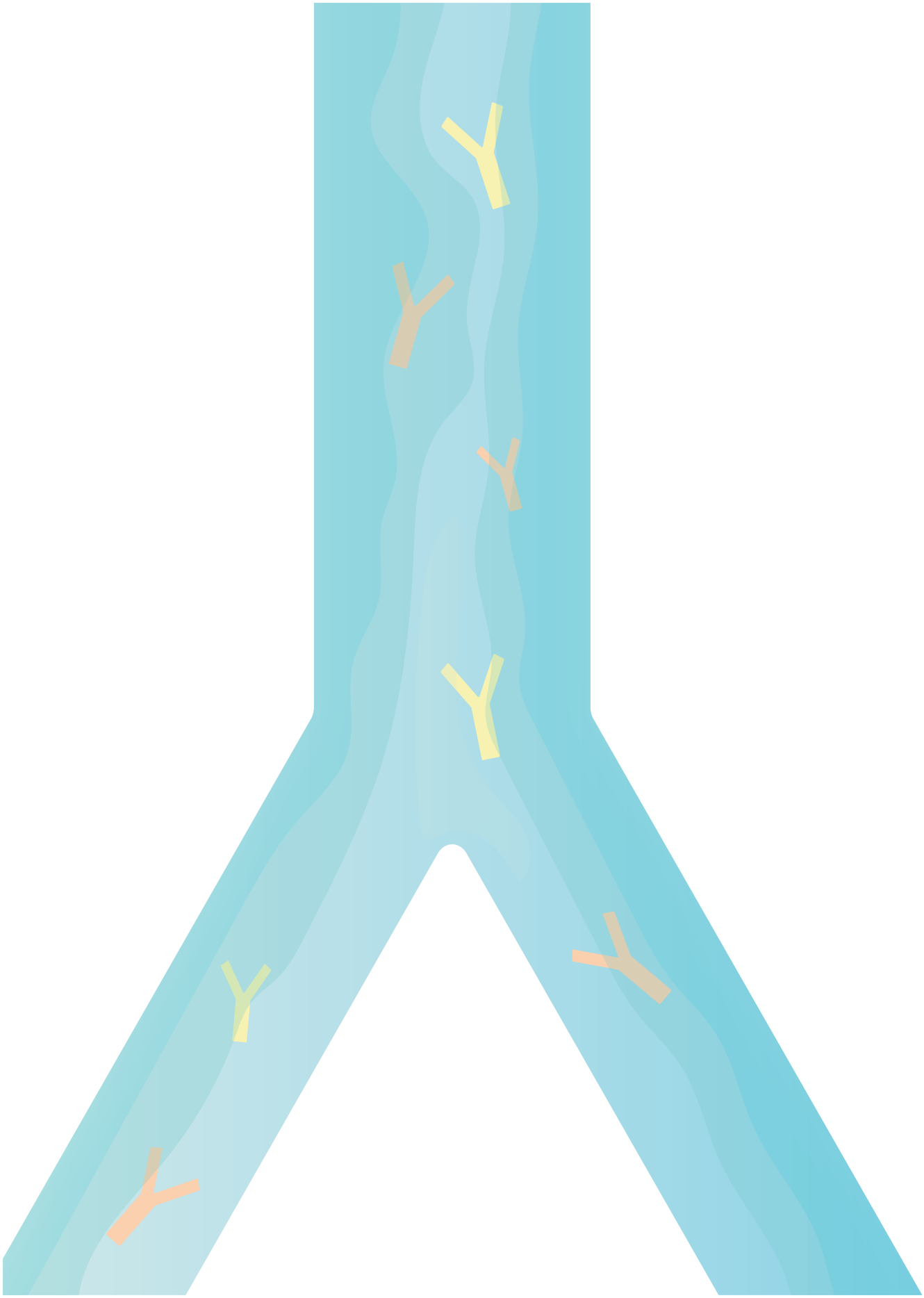
Verwoerd A, Hijdra D, Vorselaars ADM, **Crommelin HA**, van Moorsel CHM, Grutters JC, Claessen AM. Infliximab therapy balances regulatory T cells, tumour necrosis factor receptor 2 (TNFR2) expression and soluble TNFR2 in sarcoidosis. *Clin Exp Immunol* 2016, 185(2): 263-270.

Jansman FG, **Crommelin HA**, van Hout FJ, Meulenbelt J. Rhabdomyolysis in Clozapine Overdose. *Drug Saf Case Rep* 2015, 2(1): 9.

Crommelin HA*, Vorselaars ADM*, Deneer VHM, Meek B, Claessen AM, Keijsers RG, van Moorsel CHM, Grutters JC. *shared first authorship. Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur Respir J* 2015, 46(1): 175-185.

Crommelin HA, Vorselaars ADM, van Moorsel CHM, Korenromp IH, Deneer VHM, Grutters JC. Anti-TNF therapeutics for the treatment of sarcoidosis. *Immunotherapy* 2014, 6(10): 1127-1143.

van Maarseveen EM, **Crommelin HA**, Mudrikova T, van den Broek MP, van Zuilen AD. Pretransplantation pharmacokinetic curves of tacrolimus in HIV-infected patients on ritonavir-containing cART: a pilot study. *Transplantation* 2013, 95(2): 397-402.



Curriculum Vitae

CURRICULUM VITAE

Heleen Annette Crommelin was born on July 30th, 1987 in Utrecht. She finished high school at "Christelijk Gymnasium Utrecht" in 2005. She started studying pharmacy at the University of Groningen where she obtained her PharmD in 2012. During her student years she was involved in competitive rowing as a coxswain at AGSR Gyas. From April 2012 she worked as a pharmacist at Deventer Hospital/Dimence. In October 2012 she started as a PhD student at the Interstitial Lung Diseases Center of Excellence at the Department of Pulmonology at St Antonius Hospital in Nieuwegein. During her research she was active as a board member of the hospital PhD club "De Promovendiclub". In November and December 2016 she visited the Pharmacy Department of the Peter MacCallum Cancer Center in Melbourne, Australia. In January 2017 she began her hospital pharmacist training program at University Medical Center Utrecht. As part of her training program she will spend the coming year at Meander Medisch Centrum in Amersfoort.

&