



Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis

Adriane D.M. Vorselaars^{1,6}, Heleen A. Crommelin^{1,2,6}, Vera H.M. Deneer², Bob Meek³, Anke M.E. Claessen³, Ruth G.M. Keijsers⁴, Coline H.M. van Moorsel^{1,5} and Jan C. Grutters^{1,5}

Affiliations: ¹Interstitial Lung Diseases Centre of Excellence, Dept of Pulmonology, St Antonius Hospital, Nieuwegein, The Netherlands. ²Dept of Clinical Pharmacy, St Antonius Hospital, Nieuwegein, The Netherlands. ³Dept of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, The Netherlands. ⁴Dept of Nuclear Medicine, St Antonius Hospital, Nieuwegein, The Netherlands. ⁵Division of Heart and Lungs, University Medical Centre Utrecht, Utrecht, The Netherlands. ⁶Both authors contributed equally.

Correspondence: Jan C. Grutters, Interstitial Lung Diseases Centre of Excellence, Dept of Pulmonology, St Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands.
E-mail: j.grutters@antoniusziekenhuis.nl

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⁻¹ infliximab. Pulmonary function, disease activity measured by ¹⁸F-fluorodeoxyglucose (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 treatment, mean improvement in forced vital capacity (FVC) was 6.6% predicted (p=0.0007), whereas in the 6 months before start of treatment, lung function decreased. Maximum standardised uptake value (SUV_{max}) of pulmonary parenchyma on ¹⁸F-FDG PET decreased by 3.93 (p<0.0001). High SUV_{max} 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 a partial response was seen in 17% of patients. No correlation between infliximab trough level (mean 18.0 µg·mL⁻¹) and initial response was found.

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



@ERSpublications

Infliximab is highly effective in refractory ¹⁸F-FDG PET positive sarcoidosis patients

<http://ow.ly/JoxhL>

Received: Sept 15 2014 | Accepted after revision: Feb 02 2015 | First published online: April 30 2015

Support statement: This study was supported by a research grant from the St Antonius Hospital innovation fund. Funding information for this article has been deposited with FundRef.

Conflict of interest: None declared.

This study is registered at www.trialregister.nl with identifier number NTR3895.

Copyright ©ERS 2015

Introduction

Sarcoidosis is a systemic disease with a wide variety of symptoms and is histologically characterised by the formation of noncaseating 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 the 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, biological agents targeted against tumour necrosis factor (TNF) have been introduced as a third-line treatment option [11]. The chimeric monoclonal anti-TNF drug infliximab (Remicade; Centocor, Inc., Malvern, 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 randomised 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 the absence of phase III RCT trials, the use of infliximab in refractory sarcoidosis is still not endorsed by healthcare insurance companies in many countries and remains off-label globally [22].

A key question remains: how to select the patients who will benefit most from this expensive therapy. Patients with high activity on ¹⁸F-fluorodeoxyglucose (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 of the 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 treatment effect in the case of sarcoidosis.

The aim of this trial was to study for the first time 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.

Material and methods

Study subjects

All sarcoidosis patients in whom infliximab therapy was initiated at St Antonius Hospital (Nieuwegein, The Netherlands), between January 2011 and April 2013, were invited to participate in this prospective, open-label cohort study. St Antonius Hospital Nieuwegein is a national tertiary referral centre for sarcoidosis. Only patients with severe sarcoidosis, unresponsive to first- and second-line treatment, or who have experienced severe side-effects from these agents (*e.g.* worsening diabetes, psychological deterioration or liver function disorders) were eligible for inclusion in the study. The diagnosis of sarcoidosis was made according to 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 ¹⁸F-FDG PET. Exclusion criteria were vaccination with live or bacterial vaccines within the previous 3 months, active or untreated latent tuberculosis, serious infections in the last 2 months, serious right ventricular heart failure, active hepatitis, history of allergic reactions to monoclonal antibodies or their fragments, opportunistic infections within the last 6 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 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⁻¹ bodyweight at weeks 0 and 2 and then every 4 weeks over a period of 6 months. Dosing of prednisone could be tapered according to the 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 1 s (FEV1) and diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (*DLCOc*)). Small fibre neuropathy was tested using the small fibre neuropathy screening list 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 $\geq 5\%$ pred and $\geq 10\%$ pred [28].

Inflammatory response

Parameters in the inflammation category included the biomarkers soluble interleukin-2 receptor (sIL-2R), angiotensin-converting enzyme (ACE) and ¹⁸F-FDG PET maximum standardised uptake value (SUV_{max}) of the pulmonary parenchyma and, if applicable, other index localisation of sarcoidosis. ¹⁸F-FDG PET imaging was performed using a Philips Gemini TF-64 combined PET/CT device (Philips Medical Systems, Eindhoven, The Netherlands). The SUV_{max} 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 SUV_{max}. 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 programme (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⁻¹.

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 the short form (SF)-36 health survey. An improvement of 10 points was considered clinically relevant.

Composite overall response

In addition we reported 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 $\geq 5\%$ pred. A decrease in biomarkers or SUV_{max} $> 40\%$ of baseline was considered a relevant improvement. Changes within the normal range of sIL-2R, ACE and SUV_{max} on ¹⁸F-FDG PET were not taken into account when gauging response. Change in SUV_{max} of the target organ was regarded as superior to a change in inflammatory biomarkers. Clinically relevant improvement in two or three categories was considered a good or excellent response, in one category as a partial response and in none of the categories as nonresponse.

Infliximab trough levels and antibodies against infliximab

Infliximab trough levels were measured using 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 neutralising antibodies against infliximab. The presence of antibodies against infliximab was determined using radioimmunoassay [30].

Analysis

Changes in values before and after 6 months of treatment were compared using two-tailed paired t-tests. Pearson's correlation coefficients (R) between parameters were calculated using linear regression. Statistical analysis was performed using the SPSS for Windows (version 22.0; IBM, Armonk, NY, USA). Graphs were created using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). $p < 0.05$ was considered significant.

Results

Study subjects

Between January 2011 and April 2013, infliximab therapy was initiated in a total of 58 active refractory sarcoidosis patients, two of whom were not included in this study (fig. 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 fibre neuropathy. The vast majority of patients suffered from chronic sarcoidosis, with a mean disease duration at initiation of 6.8 years and the use of at least two immunosuppressant drugs prior to infliximab initiation in 92.9% of patients. Furthermore, patients had signs of high disease activity, with a mean SUV_{max} of 6.6 on ^{18}F -FDG PET in the pulmonary parenchyma, a high sIL-2R of $8824 \text{ pg}\cdot\text{mL}^{-1}$ (normal value $<3000 \text{ pg}\cdot\text{mL}^{-1}$) and a high ACE of $89.7 \text{ U}\cdot\text{L}^{-1}$ (normal value $<68 \text{ U}\cdot\text{L}^{-1}$) at start of therapy. Baseline SUV_{max} on ^{18}F -FDG PET did not correlate with Scadding stages 0–III versus Scadding stage IV (6.3 versus 6.8, respectively; $p=0.76$).

Organ function

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

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

Prior to initiation of infliximab, stable or deteriorating pulmonary function was seen in the total cohort (fig. 2b). Using repeated measurement ANOVA, FVC after 26 weeks of treatment was found to be significantly higher than at initiation of treatment and than at 6 months before treatment ($p<0.0001$ and $p=0.007$, respectively). Baseline pulmonary function tests did not predict outcome (data not shown).

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

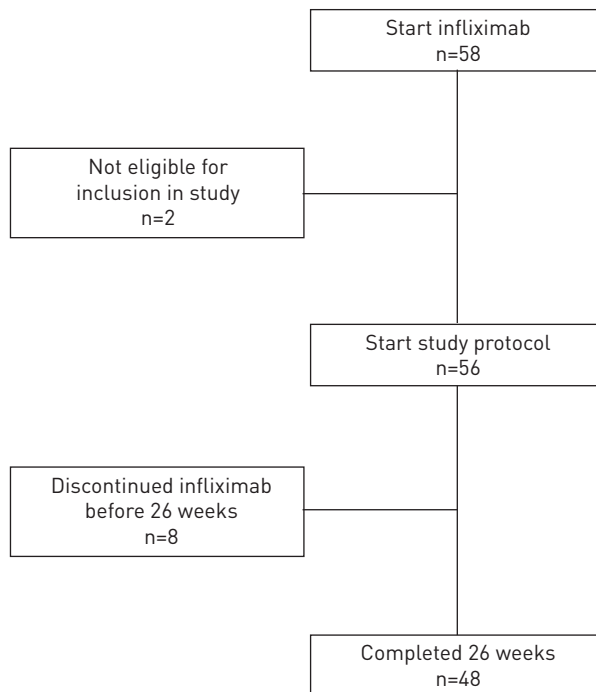


FIGURE 1 Flowchart of patient inclusion. Two patients were excluded from the study protocol, one due to incapability to give informed consent based on mental retardation and one because his cold agglutinin disease would not permit routine sampling at every visit.

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)
Main treatment indication	
Pulmonary	34 (60.7)
Cardiac	2 (3.6)
Small fibre neuropathy	8 (14.3)
Cutaneous	4 (7.2)
Central nervous system	3 (5.4)
Sinus	1 (1.8)
Myositis	1 (1.8)
Vocal cord paralysis	1 (1.8)
Ossal	1 (1.8)
Hypercalcaemia	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 (% pred)	3.32 (78.8)
FEV ₁ L (% pred)	2.30 (66.8)
DL _{CO} L (% pred)	6.01 (59.8)
δMWD m (% pred)	460.4 (62.2)
Measurements of disease activity and severity	
SUV _{max} lung parenchyma	6.6±5.3
SUV _{max} mediastinum	5.7±3.2
SUV _{max} total (including index localisation)	9.0±5.2
ACE U·L ⁻¹	89.73±49.7
ACE Z-score	4.26±4.8
sIL-2R pg·mL ⁻¹	8824±8503

Data are presented as n (%) or mean±SD, unless otherwise stated. TNF: tumour necrosis factor; FVC: forced vital capacity; % pred: % predicted; FEV₁: forced expiratory volume in 1 s; DL_{CO}: diffusing capacity for carbon monoxide corrected for haemoglobin; δMWD: 6-min walking distance; SUV_{max}: maximum standardised uptake value on ¹⁸F-fluorodeoxyglucose by positron emission tomography; ACE: angiotensin-converting enzyme; sIL-2R: soluble interleukin-2 receptor.

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

	Baseline	Change after infliximab treatment
Pulmonary function parameters		
FVC % pred	73.6	+6.6
FEV ₁ % pred	55.8	+5.8
DLCOc % pred	56.6	+4.1
6MWD % pred	61.0	+4.2
Disease activity and severity measurements		
SUV _{max} lung parenchyma	9.0±5.0	-5.3±5.6
SUV _{max} mediastinum	5.9±3.3	-2.7±3.8
SUV _{max} index localisation	9.8±5.3	-5.5±5.6
ACE U·L ⁻¹	86.2±46.3	-21.8±43.3
ACE Z score	3.7±3.9	-1.78±3.33
sIL-2R pg·mL ⁻¹	7631±4259	-3955±3883

n=28. Data are presented as mean±SD, unless otherwise stated. FVC: forced vital capacity; % pred: % predicted; FEV₁: forced expiratory volume in 1 s; DLCOc: diffusing capacity for carbon monoxide corrected for haemoglobin; 6MWD: 6-min walking distance; SUV_{max}: maximum standardised uptake value on ¹⁸F-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⁻¹) was 47 (84%). Due to use of ACE inhibitors, data on ACE levels were only usable in 49 patients, 30 (61%) of whom had levels >68 U·L⁻¹. When measuring disease activity by means of ¹⁸F-FDG PET scan before and after 26 weeks of treatment we found a decrease in SUV_{max} of mediastinum and lung parenchyma of 2.97 (p<0.0001) and 3.93 (p<0.0001), respectively. Moreover, the SUV_{max} of lungs and index localisation (e.g. heart) decreased significantly by 5.76 (p<0.0001) (fig. 3).

Both serum markers ACE and sIL-2R decreased significantly by 28.2 U·L⁻¹ (p=0.0003) and 4269.4 pg·mL⁻¹ (p<0.0001), respectively. Interestingly, ACE was higher in patients with an extrapulmonary treatment indication compared to patients with a pulmonary treatment indication (97.8 and 86.2 U·L⁻¹, respectively).

Furthermore, we found significant correlations between the change in pulmonary function and level of disease activity, indicating that pulmonary function improved for the majority of patients with the highest disease activity. In patients with a pulmonary treatment indication, improvement in FVC following treatment correlated best with SUV_{max} of the pulmonary parenchyma before treatment initiation, having a correlation coefficient of 0.62 (p=0.0004) (fig. 4). Linear regression analysis, including the parameters

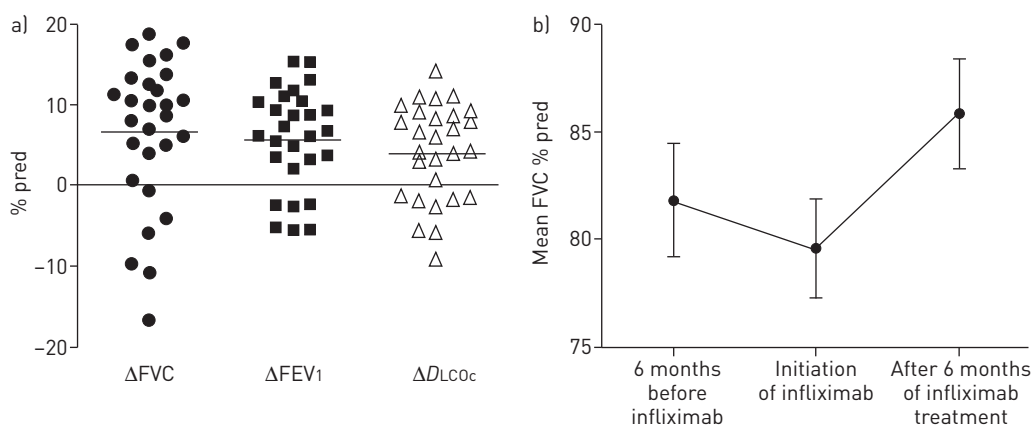


FIGURE 2 Pulmonary response in sarcoidosis. a) Change in pulmonary function after 26 weeks of infliximab therapy in patients with a pulmonary treatment indication (change in % predicted); b) mean forced vital capacity (FVC) 6 months before initiation and during 26 weeks of infliximab therapy in the total cohort (% pred). Bars represent SEM. FEV₁: forced expiratory volume in 1 s; DLCOc: diffusing capacity of the lung for carbon monoxide corrected for haemoglobin.

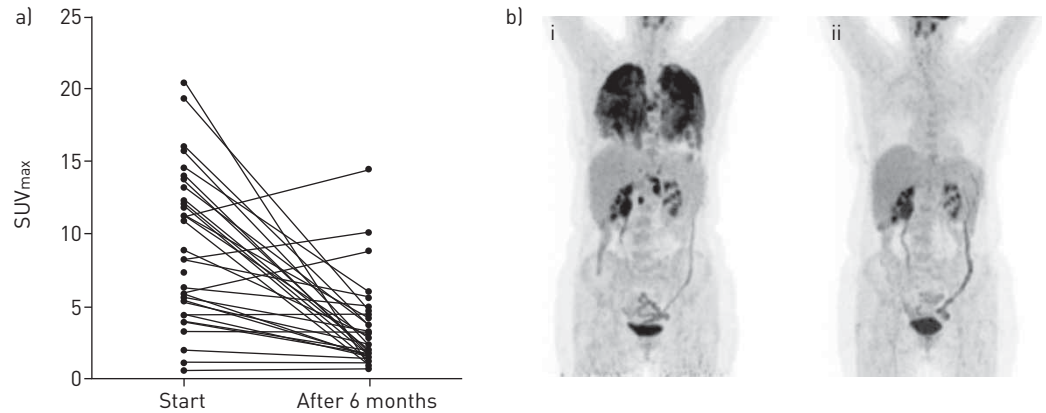


FIGURE 3 ^{18}F -fluorodeoxyglucose (FDG) by positron emission tomography (PET) activity during treatment. a) Maximum standardised uptake value (SUV_{max}) on ^{18}F -FDG PET of the target organ at initiation and after 26 weeks of infliximab treatment. b) Example of ^{18}F -FDG PET in a patient with pulmonary sarcoidosis i) before and ii) after 26 weeks of infliximab treatment.

SUV_{max} of the parenchyma and FVC at treatment initiation, predicted that FVC would improve by 1.1% per unit SUV_{max} of the parenchyma at start of therapy. Consequently, a SUV_{max} of 10 in the parenchyma at initiation predicts an FVC increase of 11% pred. Baseline sIL-2R correlated with improvement in DLCO (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 (fig. 2a) to the other patients, SUV_{max} of the pulmonary parenchyma and sIL-2R at baseline were found to be significantly different. No significant correlation with ACE, PFTs, age, sex, Scadding stage, ethnicity or disease duration was found.

Prednisone was used concurrently in 19 patients at the 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.

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.

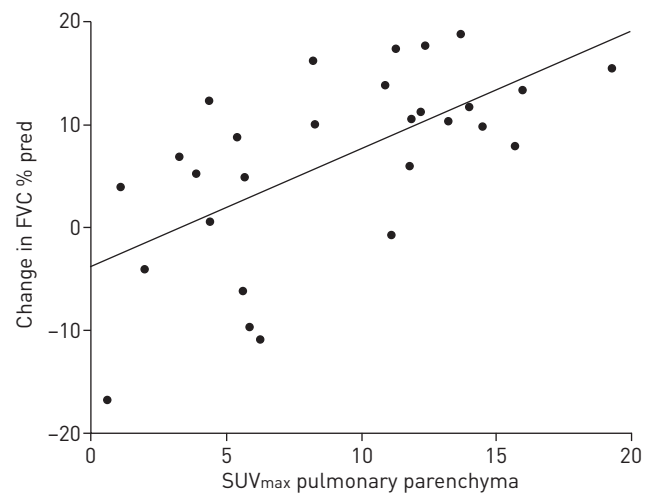


FIGURE 4 ^{18}F -fluorodeoxyglucose (FDG) by positron emission tomography (PET) activity and improvement in pulmonary function. Correlation between high activity of pulmonary parenchyma on ^{18}F -FDG PET (maximum standardised uptake value (SUV_{max})) at baseline and improvement in forced vital capacity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

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 (fig. 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.

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 $18.0 \mu\text{g}\cdot\text{mL}^{-1}$. No significant correlation between trough level and response was found. Patients with excellent or good response had a mean trough level of $18.5 \mu\text{g}\cdot\text{mL}^{-1}$, partial responders a mean trough level of $17.4 \mu\text{g}\cdot\text{mL}^{-1}$ and nonresponders a mean trough level of $27.5 \mu\text{g}\cdot\text{mL}^{-1}$.

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 1 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 $20 \text{ mg}\cdot\text{day}^{-1}$, one patient methotrexate $7.5 \text{ mg}\cdot\text{week}^{-1}$ and one patient was on prednisone $10 \text{ mg}\cdot\text{day}^{-1}$ and methotrexate $7.5 \text{ mg}\cdot\text{week}^{-1}$.

Side-effects and discontinuation of therapy

Severe side-effects were pneumonia, requiring hospitalisation and discontinuation of therapy in three patients, one of whom was hospitalised in the intensive care unit after two infusions and one other who was hospitalised after three infusions and eventually died of respiratory failure. One patient was hospitalised 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.

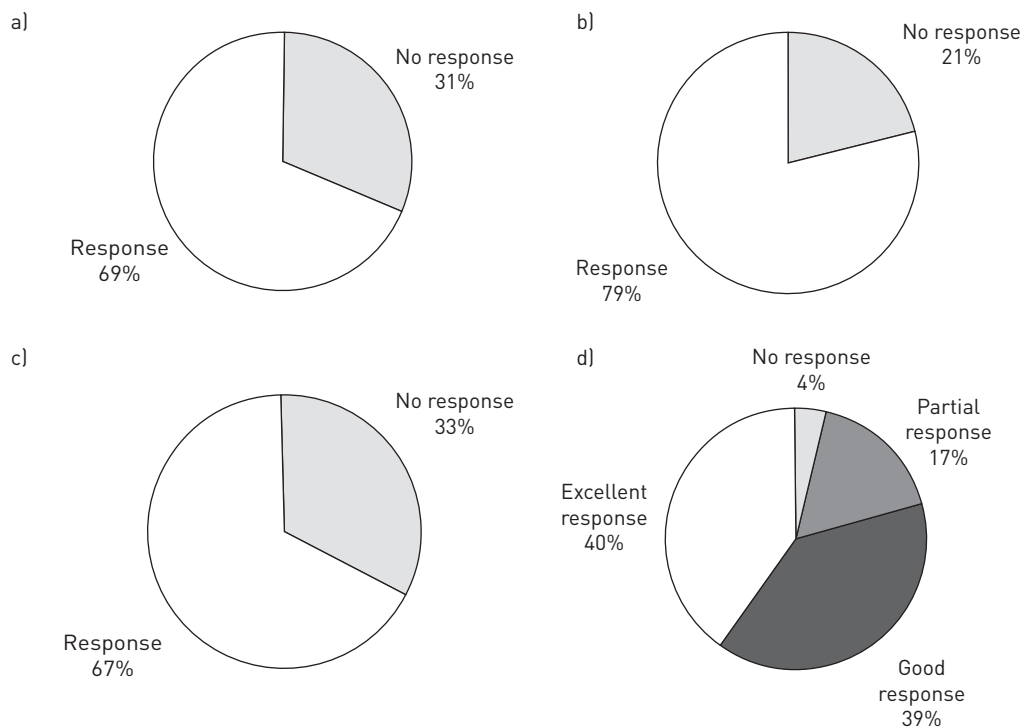


FIGURE 5 Response after 26 weeks of infliximab therapy. Response was measured in a) organ function, b) inflammation and c) quality of life; d) composite overall response. Excellent responders showed marked improvement in all three categories; good responders in two categories; and partial responders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

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 hospitalisation. Other side-effects were mild, such as headache (n=2), dizziness (n=1), oedema (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% pred 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% pred in FVC, and no treatment benefit on other major secondary clinical end-points [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 $89.7 \text{ U}\cdot\text{L}^{-1}$ and $8824 \text{ pg}\cdot\text{mL}^{-1}$, respectively (with upper limit of normal for reference values $68 \text{ U}\cdot\text{L}^{-1}$ and $3000 \text{ pg}\cdot\text{mL}^{-1}$, respectively). These values are clearly higher than ACE at initiation of the large RCT of $47.4 \text{ U}\cdot\text{L}^{-1}$ [18], or described in a recent retrospective cohort of $20.7 \text{ U}\cdot\text{L}^{-1}$ (upper limit of normal $25 \text{ U}\cdot\text{L}^{-1}$ in this study) [31]. In the latter study, mean sIL-2R at start of therapy was $3073 \text{ pg}\cdot\text{mL}^{-1}$. 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 *versus* 6 weeks). Infliximab levels were higher in our study than in the RCT (18.0 *versus* $7.5 \mu\text{g}\cdot\text{mL}^{-1}$) 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 of infliximab in sarcoidosis did not show a difference between the groups treated with 3 and $5 \text{ mg}\cdot\text{kg}^{-1}$ [17]; however, overall improvement was much lower in this RCT than in our study. A different dosing regimen could possibly further minimise 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 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 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 these patients with severe active sarcoidosis outweighs the costs, as treatment with the biological drug infliximab is over 10 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 care and the response to the individual categories depicted in fig. 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 have been unsuccessful [43], infliximab remains the best option for the group of patients 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 Centre 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, *et al.* 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; CD001114.
- 6 Vorselaars AD, van Moorsel CH, Deneer VH, *et al.* 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, *et al.* 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, *et al.* Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* 2013; 144: 805–812.
- 11 Drent M, Cremers JP, Jansen TL, *et al.* 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, *et al.* 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, *et al.* 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 Hogezaand R, *et al.* 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, *et al.* Changes in disease activity, lung function and quality of life in patients with refractory sarcoidosis after anti-TNF treatment. *Expert Opin Orphan Drugs* 2013; 1: 437–443.
- 16 Hostettler KE, Studler U, Tamm M, *et al.* Long-term treatment with infliximab in patients with sarcoidosis. *Respiration* 2012; 83: 218–224.
- 17 Baughman RP, Drent M, Kavuru M, *et al.* 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, *et al.* 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, *et al.* Sarcoidosis. *Lancet* 2014; 383: 1155–1167.
- 20 Maneiro JR, Salgado E, Gomez-Reino JJ, *et al.* 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 Vaz Carneiro A, Costa J. A prescrição fora das indicações aprovadas (off-label): prática e problemas [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, *et al.* Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur Respir J* 2014; 43: 602–609.
- 24 Garcês 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, *et al.* 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, *et al.* Established and experimental medical therapy of pulmonary sarcoidosis. *Eur Respir J* 2013; 41: 1424–1438.
- 29 Wolbink GJ, Voskuyl AE, Lems WF, *et al.* 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, *et al.* 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, *et al.* Association of the TNF- α 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, *et al.* FDG PET for gauging of sarcoid disease activity. *Semin Respir Crit Care Med* 2014; 35: 352–361.
- 34 Keijsers RG, Verzijlbergen FJ, Oyen WJ, *et al.* 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, *et al.* 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, *et al.* 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, *et al.* 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, *et al.* 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, *et al.* 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, *et al.* 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, *et al.* Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. *Eur Respir J* 2014; 44: 1296–1307.