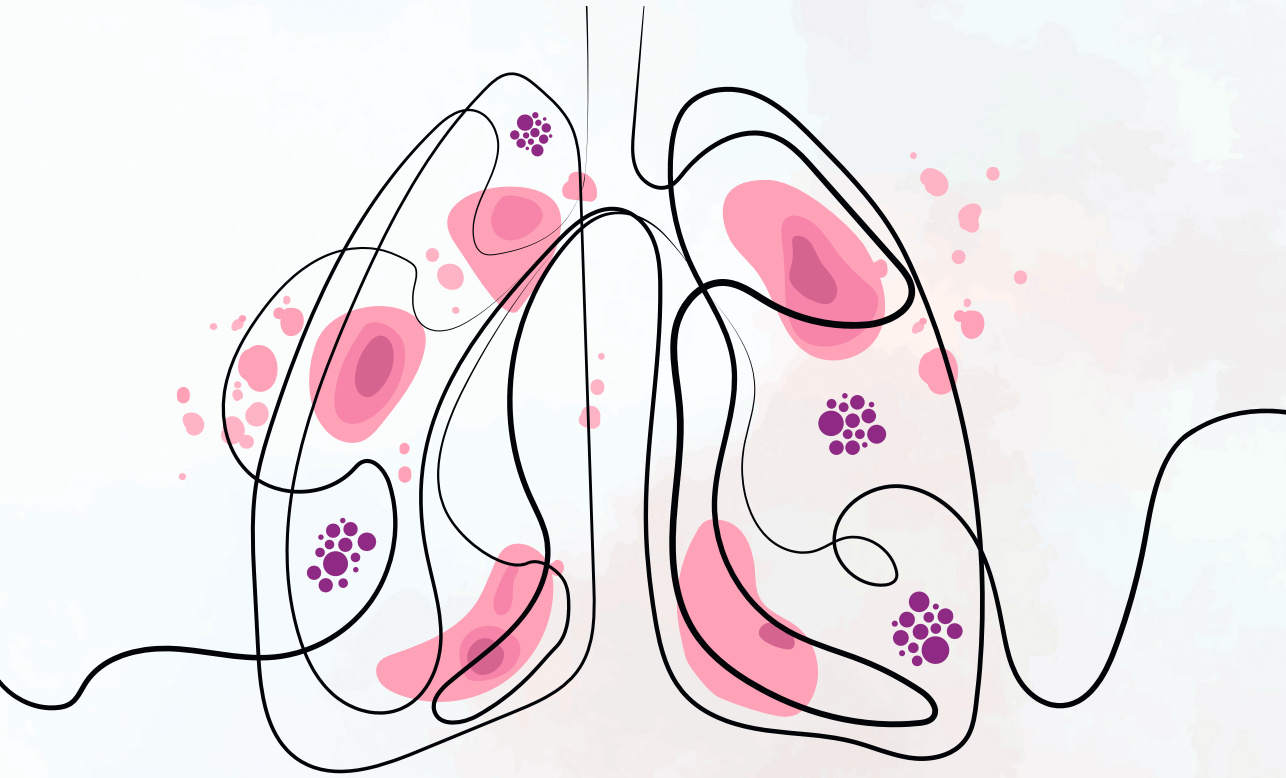
BIOMARKERS AND PHENOTYPE CONCEPTS IN SARCOIDOSIS CARE



MILOU SCHIMMELPENNINK

BIOMARKERS AND PHENOTYPE CONCEPTS IN SARCOIDOSIS CARE

MILOU SCHIMMELPENNINK

Biomarkers and phenotype concepts in sarcoidosis care
M.C. Schimmelpennink

ISBN: 978-94-6483-789-6
DOI: https://doi.org/10.33540/2085
Cover design & layout: Marilou Maes, persoonlijkproefschrift.nl
Printing: Ridderprint | www.ridderprint.nl

Parts of the research described in this thesis were financially made possible by the TopZorg ILD Grant funded by the ZonMw.

Publication of this thesis was financially supported by the Board of Directors of St. Antonius Hospital, Boehringer Ingelheim bv, Westfalen Medical bv, and Sarcoïdose.nl.

Biomarkers and phenotype concepts in sarcoidosis care

Biomarkers en fenotype concepten in de zorg voor patiënten met sarcoïdose

(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. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

dinsdag 12 maart 2024 des ochtends te 10.15 uur

door

Milou Chantal Schimmelpennink

geboren op 26 december 1990
te Abcoude

Promotor:

Prof. dr. J.C. Grutters

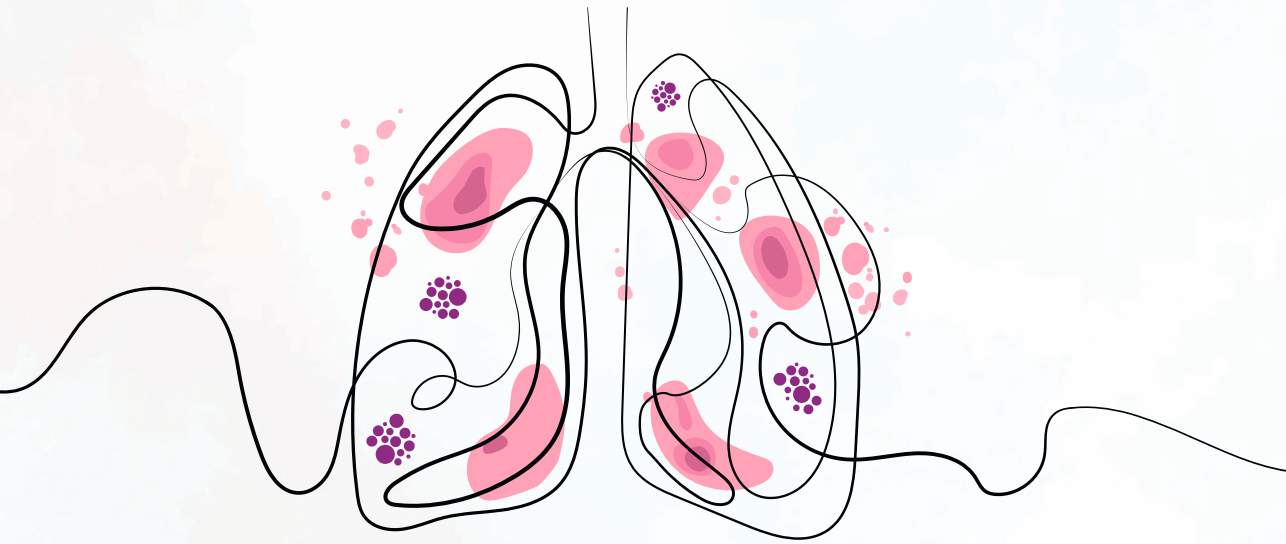
Copromotoren:

Dr. M. Veltkamp
Dr. A.D.M. Vorselaars

Beoordelingscommissie:

Prof. dr. R.A.M.J. Damoiseaux (voorzitter)
Prof. dr. P.A. de Jong
Prof. dr. H.G.M. Heijerman
Prof. dr. M.G.E.H. Lam
Prof. dr. M.S. Wijsenbeek-Lourens

TABLE OF CONTENTS



CHAPTER 1

INTRODUCTION

Sarcoidosis can be difficult to diagnose due to the heterogeneity in disease presentation and a lack of sensitive and specific diagnostic tests. Also, the disease course of sarcoidosis is unpredictable, varying from spontaneous resolution to a progressive disease course with pulmonary fibrosis, which makes clinical management challenging. Better understanding of classic and potential new biomarkers (biological markers) and phenotypes could help clinicians to guide patients with sarcoidosis regarding diagnosing, prognostication, initiation of treatment and its evaluation. The aim of this thesis is therefore to further define the role of existing biomarkers as well as identifying new biomarkers in different phenotypes of patients with sarcoidosis in order to improve clinical care in this heterogeneous complex disease.

This chapter provides an introduction into sarcoidosis and its epidemiology, aetiology, pathogenesis, clinical phenotypes and treatment. Moreover, the definition of biomarkers and their use are given as well as an overview of currently used biomarkers in sarcoidosis.

SARCOIDOSIS

Sarcoidosis is a complex granulomatous disease affecting intrathoracic lymph nodes and/or the lungs in more than 90% of the patients, although almost any organ can be affected[1]. Sarcoidosis is diagnosed when the clinical and radiological features match the histopathological evidence of non-caseating granulomas and other causes of granulomatous inflammation are excluded[2]. The most commonly reported symptoms in sarcoidosis are dyspnoea and fatigue, however a wide range of symptoms can be associated with the disease depending on which organs are involved[3,4]. The clinical course of sarcoidosis ranges from self-limiting disease to chronic course of disease requiring long- term treatment[5,6].

EPIDEMIOLOGY

Sarcoidosis is a relatively rare disorder. Worldwide the incidence varies considerably depending on ethnicity, age and gender. In countries in Northern Europe the incidence of sarcoidosis varies from 7 to 19 per 100 000 per year[7]. The lowest incidence rates are reported in Asian countries as Japan and Korea[8,9]. In the Netherlands the estimated incidence of sarcoidosis is 20 per 100 000 inhabitants[10]. However, these rates are probably an underestimation of the actual incidence, for the reason that the disease can be present without any symptoms. Sarcoidosis is diagnosed at all ages, although

the disease is very rare in children[11]. Disease onset typically occurs in the third and fourth decade and a second peak incidence is seen in women over 50 years of age[12].

AETIOLOGY AND PATHOGENESIS

Despite extensive research over the last decades, the aetiology of sarcoidosis remains largely unresolved, impeding research addressing biomarkers, clinical management and treatment of sarcoidosis[13]. The general hypothesis is that the disease originates from an exaggerated immune response to an unknown antigen in combination with genetic susceptibility. Several antigens have been proposed as trigger for sarcoidosis, such as: metals (for example beryllium, aluminium, zirconium), silica, vimentin, Propionibacteria and mycobacteria[14,15]. Besides an antigenic trigger, genetic susceptibility plays a role in the pathogenesis of sarcoidosis. Having a first-degree family member with sarcoidosis increases the risk of sarcoidosis with fourfold[16].

The pathogenesis of sarcoidosis is characterised by the clonal expansion of CD4+ T cells at sites of disease activity[17]. Presentation of the sarcoid antigen by the major histocompatibility complex (MHC) leads to proliferation of CD4+ T cell lymphocytes. The CD4+ T cell lymphocytes differentiate into different T helper cell subsets. In sarcoidosis, alveolitis is especially dominated by T helper 1 (Th1) and Th17 subsets[18–22]. Important cytokines involved in the pathogenesis of sarcoidosis are interferon gamma (IFN-γ), interleukin 2 (IL-2), interleukin 12 (IL-12) and tumour necrosis factor alpha (TNF-α)[23,24]. Under the influence of chemokines, T lymphocytes migrate to sarcoid lesions and stimulate formation of granulomas, which are aggregations of mainly macrophages and T cells[25]. Figure 1 shows an example of a non-caseating granuloma.

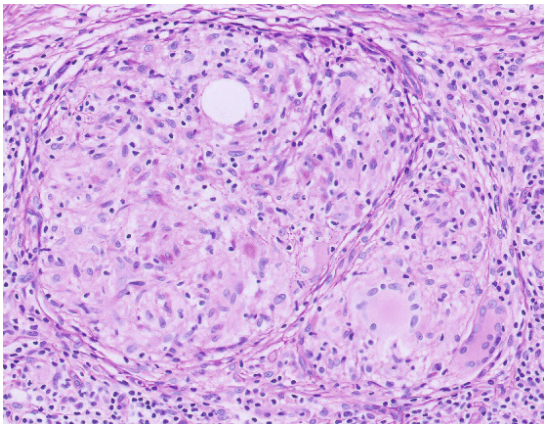


Figure 1. Non-caseating granuloma from a biopsy of a lymph node of a sarcoidosis patient.

CLINICAL PHENOTYPES

As the course of sarcoidosis is unpredictable with variable clinical outcomes, phenotyping is considered important for advancing our fundamental understanding of the disease. A phenotype is defined as the observable expression of the genotype of a patient and is considered as an interaction between the environment and the genetic background[26,27]. Phenotyping in sarcoidosis is conceptual as both the genetic background and the environmental influences remain mostly unclear. Sarcoidosis likely encompasses different phenotypes, with acute type sarcoidosis with sudden onset and high inflammatory activity (i.e. Löfgren's syndrome) at one side of the spectrum and the chronic fibrotic phenotype on the other side.

Löfgren's syndrome is the most well-defined phenotype in sarcoidosis, which was first described by Sven Löfgren in the 20th century[28–30]. The highest incidence of this phenotype is found in Scandinavia, whereas this phenotype is extremely rare in Japan[31]. This syndrome, also described as the acute form of sarcoidosis, is a benign clinical phenotype characterised by the clinical triad of bihilar lymphadenopathy, ankle arthritis and erythema nodosum[32]. In terms of genetics, variation in the human leukocyte antigen (HLA) region is linked to Löfgren's syndrome, more specifically carrying of the *HLA-DRB1*03* allele is a risk factor for Löfgren's syndrome and is associated with a benign disease course[33]. When a patient presents with Löfgren's syndrome histopathological evidence is not needed. In up to 90% of the *DRB1*03* positive patients with Löfgren's syndrome the disease resolves spontaneously[33].

Between 10%-30% of the patients develop a chronic course of disease[5]. Ongoing chronic granulomatous inflammation can cause transformation to unresolvable fibrosis. The presence of fibrosis in sarcoidosis is associated with a poor prognosis[34,35]. In addition, fibrosis can lead to severe complications, such as pulmonary hypertension and severe pulmonary infections[36,37]. A typical usual interstitial pneumonia (UIP) pattern with honeycombing and reticular abnormalities with basal predominance, although extremely rare, also appears to exist in sarcoidosis[38]. Early recognition of the fibrosing phenotype is important for prognostic and therapeutic reasons. In the next paragraph the different therapeutic options in sarcoidosis will be discussed.

TREATMENT OF SARCOIDOSIS

As the aetiology of sarcoidosis is largely unresolved, treatment of sarcoidosis is not curative but aims to suppress inflammatory activity in order to alleviate symptoms

and to prevent (further) organ damage. In pulmonary sarcoidosis, systemic anti-inflammatory therapy is required in around half of the patients[39], especially in patients with severe lung function deterioration[40]. Additionally, other treatment indications are clinically relevant cardiac sarcoidosis and clinically significant neurosarcoidosis[41]. In some patients fatigue, chronic pain or small fibre neuropathy persist, while disease activity can not be measured, deteriorating their health-related quality of life[42]. In patients with impaired quality of life and increased inflammatory activity, anti-inflammatory therapy may also be considered[40,41].

First-line anti-inflammatory drugs in sarcoidosis are corticosteroids[41]. However, corticosteroids have important long-term side effects such as osteoporosis, obesity and insulin-resistance. In therapy-resistant patients or when patients suffer from toxicity due to corticosteroids, methotrexate or azathioprine can be initiated as second-line therapy. Methotrexate and azathioprine seem equally effective as second-line treatment in patients with sarcoidosis[43].

Moreover, in refractory sarcoidosis patients tumour necrosis factor alpha inhibitors, such as infliximab and adalimumab, have been proven to be effective and safe[44–46]. More recently, biosimilars have entered the market and have been studied as less expensive alternatives in other diseases such as rheumatoid arthritis and psoriasis[47,48]. Biosimilars are biotherapeutic products that are similar in terms of efficacy and safety to its reference product.

Eventually, in a minority of the patients with pulmonary sarcoidosis granulomatous inflammation evolves into unresolvable fibrosis[5,34]. Until recently, antifibrotic therapy was only prescribed in patients with idiopathic pulmonary fibrosis (IPF). However, large trials investigating antifibrotic therapy in patients with interstitial lung disease (ILD) other than IPF shed new light on the therapeutic effect in these groups. For example, the INBUILD trial has recently demonstrated that antifibrotic therapy might be an option for patients with progressive fibrosing ILDs including some cases of sarcoidosis[49].

In order to make clinical decisions regarding therapy, the use of biomarkers might be beneficial. The next paragraph will therefore discuss the currently most frequent studied and used biomarkers in sarcoidosis.

BIOMARKERS IN SARCOIDOSIS

In this paragraph first the definition of a biomarker is given. Next, the different purposes and qualities of biomarkers are described in general. Subsequently, a more detailed introduction is given on serological, lung function, bronchoalveolar lavage (BAL) fluid and radiological biomarkers and genetic variants which have been studied and used in clinical management of sarcoidosis.

Definition, purposes and qualities

A biological maker (biomarker) is to be defined as "a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[50].

Biomarkers can serve different purposes, for example to diagnose a disease, for prognostication, for predicting clinical outcomes in case of therapy, to monitor disease activity or evaluate treatment response. These different purposes are discussed in the next paragraphs.

A *diagnostic biomarker* detects or confirms the presence of a disease or condition of interest, or identifies an individual with a subtype of the disease[51]. Subsequently, another purpose of a diagnostic biomarker is to identify an individual with a subtype of the disease (phenotype)[51]. To date, no single marker has been found that can confirm the diagnosis of sarcoidosis and has perfect clinical and analytical performance. As sarcoidosis is a complex disease, it is plausible that a combination of biomarkers will perform better in diagnosing and prognosing sarcoidosis than a single biomarker[52]. Furthermore, to improve the diagnostic process in sarcoidosis, a deeper understanding is needed in the role of the currently used biomarkers. Lastly, there is a need for diagnostic biomarkers that can differentiate between various phenotypes of sarcoidosis.

A *prognostic biomarker* is used to identify the likelihood of a clinical event, disease recurrence, or disease progression in patients with a disease or medical condition of interest[51]. Prognostication serves several important purposes. Firstly, prognostication is important to raise understanding in the underlying biologic process of the disease. Secondly, prognostication is important for clinical decision making. Lastly, prognostication is important for patients and their relatives to provide accurate information on their potential disease course[53]. In sarcoidosis prognostic biomarkers are urgently needed given the diffuse variety in clinical outcomes.

A *predictive biomarker* is defined by the finding that the presence or change in the biomarker predicts an individual or group of individuals more likely to experience a favourable or unfavourable effect from the exposure to a medical product or environmental agent[51]. In sarcoidosis, careful patient selection for therapy utilising predictive biomarkers may prevent from overtreatment and thereby minimises the risk of toxicity of treatment and other negative side effects, for example obesity in patients treated with corticosteroids.

A *monitoring biomarker*, is a biomarker that can be measured serially to assess the status of a disease or medical condition for evidence of exposure to a medical product, or to detect a therapeutic effect[51]. Monitoring biomarkers play a pivotal role in the assessment of disease activity, tracking of disease progression, and evaluation of therapy response. In sarcoidosis, there is a need for monitoring biomarkers to guide clinical decisions regarding therapy.

Finally, the evaluation of the performance of biomarkers needs to be introduced. The accuracy of a biomarker is reflected by the sensitivity and specificity. Sensitivity of a test is defined as "the proportion of true negatives that are correctly identified by the test"[54]. Specificity is defined as "the proportion of true negatives that are correctly identified by a test"[54]. The sensitivity and specificity of a biomarker depends on the cut-off value. The ideal cut-off value of a biomarker can be determined with the area under the curve of a receiver operating curve (ROC curve)[55].

The next paragraphs will discuss the most studied and clinically used serological, lung function, BAL fluid and radiological biomarkers and genetic variants in sarcoidosis.

Biomarkers in sarcoidosis

Serological biomarkers

Angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) are probably the two most studied and clinically used serological biomarkers in sarcoidosis worldwide. ACE converts angiotensin I in angiotensin II, and thereby contributes to the renin-angiotensin system[56]. In sarcoidosis, levels of ACE can be increased because this enzyme is released by the epithelioid cells of granulomas[57]. The sensitivity and specificity of ACE for the diagnosis sarcoidosis are both limited[58–60]. In addition, the insertion/deletion polymorphism of the ACE gene causes inter-individual variability, which also affects the sensitivity of ACE for diagnosing sarcoidosis[61]. Although the diagnostic value of ACE is limited, sequential measurement of ACE is useful to monitor disease activity in the follow-up of sarcoidosis[62,63].

Next to ACE, sIL-2R is the second most used and studied biomarker in sarcoidosis. sIL-2R is produced by activated lymphocytes[64]. Although sensitivity and specificity of sIL-2R varies, studies suggest a potential role as diagnostic marker which may also discriminate between phenotypes of sarcoidosis[52]. Moreover, sIL-2R correlates with lymphocytosis in BAL fluid, which indicates that it is a marker of disease activity in sarcoidosis[65]. Less is known about the diagnostic value of sIL-2R to differentiate sarcoidosis from other ILD. Lastly, the prognostic value of sIL-2R as marker for chronicity in sarcoidosis is not well established.

Lung function

Spirometry and diffusing capacity for carbon monoxide (DLCO) are routinely performed in all patients with pulmonary involvement at time of work-up for diagnosis and in the follow-up to detect functional worsening or improvement.

In sarcoidosis, both obstructive as well as restrictive lung function patterns can be found with spirometry[66]. An obstructive type of ventilatory impairment, defined as a diminished forced expiratory volume in one second (FEV1), can be caused at different levels: at laryngeal level, compression of the central airways and small airway disease[67,68]. A restrictive type of ventilatory pattern, defined as a reduced total lung capacity (TLC) and a reduced forced vital capacity (FVC), is typically found in patients with pulmonary fibrosis[69]. Nevertheless, in sarcoidosis DLCO is more often affected than the lung volumes (such as FVC and FEV1)[70]. A diminished gas transfer rendered by a decreased DLCO indicates abnormality of the interstitium, which can be explained by granulomatous alveolitis or small airway involvement. In patients with advanced pulmonary sarcoidosis DLCO can also be reduced due to pulmonary hypertension[71]. Another cause of reduction of DLCO is heart failure[72]. As sarcoidosis can influence the different lung functional indices the composite physiologic index (CPI) introduced by Wells[73], encompassing the FVC, FEV1 and DLCO, might give a more accurate reflection of the lung functional status in sarcoidosis. Subsequently, CPI has proven to be a prognostic marker in sarcoidosis[74,75].

Bronchoalveolar lavage

BAL can be used in the diagnostic work-up of sarcoidosis to support the diagnosis when biopsy is not possible due to various reasons (e.g. clinical condition, preference of patients). Patients with sarcoidosis typically present with a lymphocytic alveolitis and an increased CD4+/CD8+ ratio. High CD4+/CD8+ ratio is associated with a favourable outcome of disease[76,77]. A low CD104+CD4+/CD8+ ratio is associated with worse prognosis[78,79].

Radiological markers

Here we will discuss three radiological markers utilized in sarcoidosis, namely chest radiographs, HRCT and ^{18}F-fluorodeoxyglucose (FDG) by positron emission tomography (PET).

Chest radiographs are often performed in sarcoidosis at diagnosis and during follow-up of patients with pulmonary involvement. In 1961 Scadding proposed a radiological classification system of chest radiographs for sarcoidosis[80]. This system was adapted by DeRemee[81], and includes the following stages: stage 0 indicating a normal radiograph, stage I indicating hilar lymphadenopathy, stage II indicating hilar lymphadenopathy and presence of parenchymal infiltrates, stage III indicating only parenchymal infiltrates, and stage IV when features of pulmonary fibrosis are present. Sarcoidosis patients do not necessarily go through the Scadding stages in sequential order when progression occurs. Nowadays, this classification system is still the most used radiological classification system in thoracic sarcoidosis despite its low sensitivity and high inter-observer variability[82]. The Scadding stages provide some prognostic information as patients with lower Scadding stages are more likely to have spontaneous resolution of disease, while Scadding stage IV is correlated to increased mortality[34]. However, the use of Scadding stages as classification system can be misleading in terms of danger/non-dangerous sarcoidosis as it does not express other major organ involvement. This is also a major limitation for the use of Scadding stages in clinical practice, resulting in using the use of Scadding stages as classification system unsuitable. Despite its limitations, the Scadding stages remain a widely utilised radiological classification system in sarcoidosis in the absence of a more suitable alternative.

In contrast to Scadding stages *high-resolution computed tomography (HRCT)* has been proven to be more sensitive[83]. Several staging systems have been proposed for sarcoidosis patterns on HRCT[74,84]. Recently, Walsh and colleagues[74] developed an integrated clinicoradiological staging system including the following radiological parameters: extent of fibrosis, groundglass, other pattern, bronchiectasis, emphysema and the ratio between main pulmonary artery diameter and the ascending aorta diameter. However, this clinicoradiological staging system needs external validation in a large observational cohort, subsequently it is not incorporated in the clinical guidelines of sarcoidosis[2,85]. Nevertheless, until now, we have no standardised staging system in sarcoidosis.

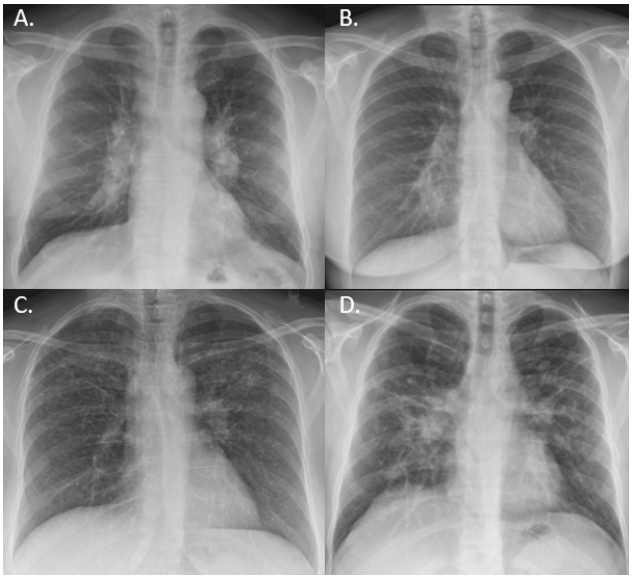


Figure 2. Example of chest radiographs of a patient with A. Scadding stage I with bilateral hilar lymphadenopathy, B. Scadding stage II with bilateral hilar lymphadenopathy and parenchymal infiltrates, C. Scadding stage III with parenchymal infiltrates and D. Scadding stage IV with fibrosis.

While chest radiographs and HRCT only render extent of disease in the chest, ^{18}F-FDG PET is useful in detecting occult and active sarcoid lesions throughout the whole body in patients with sarcoidosis[86]. Maximum standardised uptake value (SUVmax) is the most commonly used semi-quantitative measure in sarcoidosis, however it only captures the highest glycolysis in one pixel.

Sensitivity of ^{18}F-FDG PET/CT for detecting active sarcoidosis varies from 80-97%[52]. ^{18}F-FDG PET can differentiate sarcoidosis from lung cancer with lymph node metastasis with a specificity of almost 70%[87]. Additionally, a systematic review demonstrated ^{18}F-FDG PET/CT can detect cardiac sarcoidosis with a pooled sensitivity of 83% and a pooled specificity of 84%[88]. Furthermore, ^{18}F-FDG PET/CT can be used as a prognostic biomarker, as high SUVmax on ^{18}F-FDG PET/CT can predict disease progression[89] and relapse after discontinuation of infliximab[90].

Adams and colleagues developed a new semi-quantitative measure, the total lung glycolysis, to assess disease activity in pulmonary sarcoidosis[91]. Nonetheless, this PET marker needs validation as a monitoring and predictive biomarker in sarcoidosis.

Genetic variants

As a last type of biomarker, genetic variants will be discussed. Over the last years, growing evidence indicates that genetic susceptibility plays an important role in the development of sarcoidosis[92]. In patients with sarcoidosis, genetic variants have been found in the HLA genes of the major histocompatibility complex class II. Variants in the HLA-DR gene are associated with different clinical phenotypes. For example, *HLA-DRB1**03 is associated with Löfgren's disease and a benign course of disease, whereas *HLA-DRB1**15 is associated with a chronic course of disease[33,93]. However, HLA typing is laborious and expensive. An alternative approach may be found in tagging *HLA-DRB1* types by single nucleotide polymorphisms (SNPs) in linkage disequilibrium with DRB1 genotypes[94], which is less time consuming. In other diseases, studies have shown that tagging of SNP rs2040410 allele A and SNP rs3135388 allele A can identify *HLA-DRB1*0301* and *HLA-DRB1*1501* with great sensitivity[95]. However, to our knowledge, no studies have validated the tag SNPs for *HLA-DRB1*03* and *-DRB1*15* in sarcoidosis.

Another genetic variant studied in interstitial lung diseases is a polymorphism in the promotor region of the *Mucin 5B (MUC5B)* gene[96–102]. For example, it is well known that this promoter polymorphism of *MUC5B* is associated with IPF[98–102], and more recent studies demonstrated that *MUC5B* is also associated with other fibrotic ILD, such as rheumatoid arthritis-associated ILD[96]. Nevertheless, in sarcoidosis, *MUC5B* was not associated with fibrosing sarcoidosis or with disease progression[102]. However, to date, the association between *MUC5B* and the presence of a UIP pattern in sarcoidosis has not yet been investigated.

THESIS OUTLINE

The aim of this thesis is to improve clinical management by providing a deeper understanding in the role of the existing biomarkers and to identify new biomarkers in different phenotypes of sarcoidosis.

Chapter 2 provides an overview of the conventional serological and BAL markers which are most studied and clinically used in sarcoidosis. Next, it will discuss potential new biomarkers.

Chapter 3 shows data on the diagnostic and prognostic value of serum sIL-2R in sarcoidosis.

Chapter 4 correlates biomarkers in BAL fluid with HLA tag single nucleotide polymorphisms in patients with Löfgren's syndrome and patients with non-Löfgren's sarcoidosis.

Chapter 5 compares TLuG, a new semiquantitative value of ^{18}F-FDG PET/CT, to SUVmax as therapeutic biomarker in patients with pulmonary sarcoidosis treated with infliximab.

Chapter 6 describes the efficacy and safety of the infliximab biosimilar Inflectra® in patients with severe sarcoidosis.

Chapter 7 defines the characteristics of a patient cohort with advanced pulmonary sarcoidosis and provides insights in the progressive fibrosing phenotype in sarcoidosis, including of *MUC5B*.

Chapter 8 provides a summary of the findings, a general discussion and the conclusion of this thesis.

REFERENCES

1. Te, H. S. *et al.* Clinical characteristics and organ system involvement in sarcoidosis: comparison of the University of Minnesota Cohort with other cohorts. *BMC Pulm. Med.* **20**, (2020).
2. Crouser, E. D. *et al.* Diagnosis and Detection of Sarcoidosis. An official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* **201**, e26–e51 (2020).
3. Bloem, A. E. M. *et al.* Respiratory and non-respiratory symptoms in patients with IPF or sarcoidosis and controls. *Heart Lung* **61**, 136–146 (2023).
4. Wirnsberger, R. M., De Vries, J., Wouters, E. F. M. & Drent, M. Clinical presentation of sarcoidosis in the Netherlands An epidemiological study. *Neth. J. Med.* **53**, 53–60 (1998).
5. Judson, M. A., Boan, A. D. & Lackland, D. T. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. *Sarcoidosis, Vasc. Diffus. Lung Dis. Off. J. WASOG* **29**, 119–127 (2012).
6. Rubio-Rivas, M. & Corbella, X. Clinical phenotypes and prediction of chronicity in sarcoidosis using cluster analysis in a prospective cohort of 694 patients. *Eur. J. Intern. Med.* **77**, (2020).
7. Cozier, Y. C., Arkema, E. V., Rodriguez, J. V., Berman, J. S. & Govender, P. Epidemiology of sarcoidosis: solving the jigsaw puzzle. *Sarcoidosis* 8–24 (2022). doi:10.1183/2312508X.10031120
8. Morimoto, T. *et al.* Epidemiology of sarcoidosis in Japan. *Eur Respir J.* **31**, 372–9 (2008).
9. Yoon, H. Y., Kim, H. M., Kim, Y. J. & Song, J. W. Prevalence and incidence of sarcoidosis in Korea: A nationwide population-based study. *Respir. Res.* **19**, (2018).
10. Mateyo, K. & Thomeer, M. Sarcoidosis around the Globe. *Semin. Respir. Crit. Care Med.* **38**, 393–403 (2017).
11. Hoffmann, A., Milman, N. & Byg, K.-E. Childhood sarcoidosis in Denmark 1979-1994: incidence, clinical features and laboratory results at presentation in 48 children. *Acta Paediatr.* **93**, 30–36 (2007).
12. Ungprasert, P. *et al.* Epidemiology of Sarcoidosis 1946-2013: A Population-Based Study. *Mayo Clin. Proc.* **91**, 183–188 (2016).
13. Spagnolo, P. & Grunewald, J. Aetiopathogenesis, molecular determinants and immunological features. in *Sarcoidosis (ERS Monograph)* 25–40 (2022).
14. Beijer, E. *et al.* Immunoreactivity to metal and silica associates with sarcoidosis in Dutch patients. *Respir. Res.* **21**, (2020).
15. Beijer, E. *et al.* Simultaneous testing of immunological sensitization to multiple antigens in sarcoidosis reveals an association with inorganic antigens specifically related to a fibrotic phenotype. *Clin. Exp. Immunol.* **203**, 115–124 (2021).
16. Rossides, M. *et al.* Familial aggregation and heritability of sarcoidosis: a Swedish nested case–control study. *Eur Respir J.* **52**, 1800385 (2018).
17. Agostini, C., Adami, F. & Semenzato, G. New pathogenetic insights into the sarcoid granuloma. *Curr. Opin. Rheumatol.* **12**, 71–76 (2000).
18. Ostadkarampour, M. *et al.* Higher levels of interleukin IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Löfgren's syndrome. *Clin Exp Immunol.* **178**, 342–52 (2014).

19. Ten Berge, B. *et al.* Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. *Rheumatol.* **51**, 37–46 (2012).

20. Broos, C. E. *et al.* Increased T-helper 17.1 cells in sarcoidosis mediastinal lymph nodes. *Eur. Respir. J.* **51**, (2018).

21. Ramstein, J. *et al.* IFN-γ-Producing T-Helper 17.1 Cells Are Increased in Sarcoidosis and Are More Prevalent than T-Helper Type 1 Cells. *Am. J. Respir. Crit. Care Med.* **193**, 1281–1291 (2016).

22. Zhang, H., Costabel, U. & Dai, H. The Role of Diverse Immune Cells in Sarcoidosis. *Front. Immunol.* **12**, (2021).

23. Wahlström, J. *et al.* Analysis of Intracellular Cytokines in CD4+ and CD8+ Lung and Blood T Cells in Sarcoidosis. *Am J Respir Crit Care Med* **163**, 115–121 (2001).

24. D R Moller, J D Forman, M C Liu, P W Noble, B M Greenlee, P Vyas, D A Holden, J M Forrester, A Lazarus, M Wysocka, G Trinchieri, C. K. Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. *J Immunol.* **156**, 4952–60 (1996).

25. Spector, W. G. Immunologic components of granuloma formation. Epithelioid cells, giant cells, and sarcoidosis. *Ann. N. Y. Acad. Sci.* **278**, 3–6 (1976).

26. Schulze, T. G. & McMahon, F. J. Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. *Hum. Hered.* **58**, 131–138 (2004).

27. Grunewald, J. *et al.* Sarcoidosis. *Nat. Rev. Dis. Prim.* **5**, (2019).

28. Löfgren, S. Primary Pulmonary Sarcoidosis. I. Early Signs and Symptoms. *Acta Med. Scand.* **145**, 424–431 (1953).

29. Löfgren, S. Primary Pulmonary Sarcoidosis. II. Clinical Course and Prognosis. *Acta Med. Scand.* **145**, 465–474 (1953).

30. Löfgren, S. & Lundbäck, H. The Bilateral Hilar Lymphoma Syndrome. A Study of the Relation to Tuberculosis and Sarcoldosis in 212 Cases. *Acta Med. Scand.* **142**, 265–273 (1952).

31. Ohta, H. *et al.* Acute-onset Sarcoidosis with Erythema Nodosum and Polyarthralgia (Löfgren's Syndrome) in Japan: a Case Report and a Review of the Literature. *Intern Med.* **45**, 659–62 (2006).

32. Karakaya, B., Kaiser, Y., van Moorsel, C. H. M. & Grunewald, J. Lofgren's Syndrome: Diagnosis, Management, and Disease Pathogenesis. *Semin. Respir. Crit. Care Med.* **38**, 463–476 (2017).

33. Grunewald, J. & Eklund, A. Löfgren's syndrome: Human leukocyte antigen strongly influences the disease course. *Am. J. Respir. Crit. Care Med.* **179**, 307–312 (2009).

34. Kirkil, G., Lower, E. E. & Baughman, R. P. Predictors of Mortality in Pulmonary Sarcoidosis. *Chest* **153**, 105–113 (2018).

35. Jeny, F. *et al.* Predictors of mortality in fibrosing pulmonary sarcoidosis. *Respir. Med.* **169**, 105997 (2020).

36. Rossides, M. *et al.* Risk of first and recurrent serious infection in sarcoidosis: a Swedish register-based cohort study. *Eur. Respir. J.* **56**, 2000767 (2020).

37. Sulica, R. *et al.* Distinctive clinical, radiographic, and functional characteristics of patients with sarcoidosis-related pulmonary hypertension. *Chest* **128**, 1483–1489 (2005).

38. Shigemitsu, H., Oblad, J. M., Sharma, O. P. & Koss, M. N. Chronic interstitial pneumonitis in end-stage sarcoidosis. *European Respiratory Journal* **35**, 695–697 (2010).

39. Baughman, R. P. *et al.* Presenting characteristics as predictors of duration of treatment in sarcoidosis. *QJM An Int. J. Med.* **99**, 307–315 (2006).
40. Baughman, R. P., Judson, M. A. & Wells, A. The indications for the treatment of sarcoidosis: Wells Law. *Sarcoidosis, Vasc. Diffus. Lung Dis.* **34**, 280 (2017).
41. Baughman, R. P. *et al.* ERS clinical practice guidelines on treatment of sarcoidosis. *Eur. Respir. J.* **58**, (2021).
42. Korenromp, I. H. E., Grutters, J. C., Van den Bosch, J. M. M. & Heijnen, C. J. Post-inflammatory fatigue in sarcoidosis: personality profiles, psychological symptoms and stress hormones. *J. Psychosom. Res.* **72**, 97–102 (2012).
43. Vorselaars, A. D. M. *et al.* Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* **144**, 805–812 (2013).
44. Baughman, R. P. *et al.* Infliximab Therapy in Patients with Chronic Sarcoidosis and Pulmonary Involvement. *Am J Respir Crit Care Med* **174**, 795–802 (2006).
45. Vorselaars, A. D. *et al.* Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur. Respir. J.* **46**, 175–185 (2015).
46. Pariser, R. J., 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.* **68**, 765–773 (2013).
47. Yoo, D. H. *et al.* A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: The PLANETRA study. *Ann. Rheum. Dis.* **72**, 1613–1620 (2013).
48. Dapavo, P., Vujic, I., Fierro, M. T., Quaglino, P. & Sanlorenzo, M. The infliximab biosimilar in the treatment of moderate to severe plaque psoriasis. *J. Am. Acad. Dermatol.* **75**, 736–739 (2016).
49. Flaherty, K. R. *et al.* Nintedanib in progressive fibrosing interstitial lung diseases. *N. Engl. J. Med.* **381**, 1718–1727 (2019).
50. Atkinson, A. J. *et al.* Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **69**, 89–95 (2001).
51. Califf, R. M. Biomarker definitions and their applications. *Exp. Biol. Med.* **243**, 213 (2018).
52. Korenromp, I. H. E., Maier, L. A. & Grutters, J. C. Serum and imaging biomarkers. in *Sarcoidosis (ERS Monograph)* 107–121 (2022).
53. Halabi, S. & Owzar, K. The Importance of Identifying and Validating Prognostic Factors in Oncology. *Semin. Oncol.* **37**, 9–18 (2010).
54. Altman, D. G. & Bland, J. M. Diagnostic tests. 1: Sensitivity and specificity. *BMJ* **308**, 1552 (1994).
55. Cook, N. Use and misuse of the receiver operating characteristic curve in risk prediction. *Am Hear. Assoc* **115**, 928–935 (2007).
56. Ryan, J. W., Smith, U. & Niemeyer, R. S. Angiotensin I: Metabolism by Plasma Membrane of Lung. *Science (80-.).* **176**, 64–66 (1972).
57. Okabe, T., Suzuki, A., Ishikawa, H., Yotsumoto, H. & Ohsawa, N. Cells originating from sarcoid granulomas in vitro. *Am. Rev. Respir. Dis.* **124**, 608–612 (1981).

58. Popevic, S. *et al.* Verifying Sarcoidosis Activity: Chitotriosidase versus ACE in Sarcoidosis - a Case-control Study. *J. Med. Biochem.* **35**, 390–400 (2016).

59. Ungprasert, P., Carmona, E. M., Crowson, C. S. & Matteson, E. L. Diagnostic Utility of Angiotensin-Converting Enzyme in Sarcoidosis: A Population-Based Study. *Lung* **194**, 91–95 (2016).

60. Eurelings, L. E. M. *et al.* Sensitivity and specificity of serum soluble interleukin-2 receptor for diagnosing sarcoidosis in a population of patients suspected of sarcoidosis. *PLoS One* **14**, e0223897 (2019).

61. Csongrádi, A. *et al.* Optimized angiotensin-converting enzyme activity assay for the accurate diagnosis of sarcoidosis. *Clin. Chem. Lab. Med.* **56**, 1117–1125 (2018).

62. Baughman, R. P., Ploysongsang, Y., Roberts, R. D. & Srivastava, L. Effects of sarcoid and steroids on angiotensin-converting enzyme. *Am. Rev. Respir. Dis.* **128**, 631–633 (1983).

63. Vorselaars, A. D. M. *et al.* ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. *Respir. Med.* **109**, 279–285 (2015).

64. Rubin, L. A. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol. (Baltimore, Md. 1950)* **135**, 3172–3177 (1985).

65. Grutters, J. C. *et al.* Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest* **124**, 186–195 (2003).

66. Calaras, D., Munteanu, O., Scaletchi, V., Simionica, I. & Botnaru, V. Ventilatory disturbances in patients with intrathoracic sarcoidosis - a study from a functional and histological perspective. *Sarcoidosis Vasc. Diffus. Lung Dis.* **34**, 58–67 (2017).

67. Udwadia, Z. F., Pilling, J. R., Jenkins, P. F., Harrison, D. W. & Harrison, B. D. W. Bronchoscopic and bronchographic findings in 12 patients with sarcoidosis and severe or progressive airways obstruction. *Thorax* **45**, 272–275 (1990).

68. Chambellan, A. *et al.* Endoluminal stenosis of proximal bronchi in sarcoidosis: Bronchoscopy, function, and evolution. *Chest* **127**, 472–481 (2005).

69. Oldham, J. M. *et al.* Lung function trajectory in progressive fibrosing interstitial lung disease. *Eur Respir J.* **59**, 2101396. (2022).

70. Boros, P. W. *et al.* Impaired lung compliance and DL, CO but no restrictive ventilatory defect in sarcoidosis. *Eur. Respir. J.* **36**, 1315–1322 (2010).

71. Baughman, R. P. *et al.* Clinical features of sarcoidosis associated pulmonary hypertension: Results of a multi-national registry. *Respir. Med.* **139**, 72–78 (2018).

72. Magini, A. *et al.* Variability in pulmonary diffusing capacity in heart failure. *Respir. Physiol. Neurobiol.* **280**, 103473 (2020).

73. Wells, A. U. *et al.* Idiopathic pulmonary fibrosis: A composite physiologic index derived from disease extent observed by computed tomography. *Am. J. Respir. Crit. Care Med.* **167**, 962–969 (2003).

74. Walsh, S. L. F. *et al.* An integrated clinicoradiological staging system for pulmonary sarcoidosis: A case-cohort study. *Lancet Respir. Med.* **2**, 123–130 (2014).

75. Tanizawa, K. *et al.* Lung function decline in sarcoidosis. *Resp Investig.* **60**, 551–561 (2022).

76. Doubková, M., Pospíšil, Z., Skřičková, J. & Doubek, M. Prognostic markers of sarcoidosis: An analysis of patients from everyday pneumological practice. *Clin. Respir. J.* **9**, 443–449 (2015).

77. Planck, A., Eklund, A. & Grunewald, J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **18**, 64–9 (2001).

78. Heron, M. *et al.* Evaluation of CD103 as a cellular marker for the diagnosis of pulmonary sarcoidosis. *Clin Immunol.* **126**, 338–44 (2008).

79. Mota, P. C. *et al.* Diagnostic value of CD103 expression in bronchoalveolar lymphocytes in sarcoidosis. *Respir. Med.* **106**, 1014–1020 (2012).

80. Scadding, J. G. Prognosis of intratitoracic sarcoidosis in england. *Br. Med. J.* **2**, 1165–1172 (1961).

81. Deremee, R. A. The Roentgenographic Staging of sarcoidosis· Historic and Contemporary Perspectives. *Chest* **83**, 128–33 (1983).

82. Baughman, R. P. *et al.* Changes in chest roentgenogram of sarcoidosis patients during a clinical trial of infliximab therapy: Comparison of different methods of evaluation. *Chest* **136**, 526–535 (2009).

83. Grenier, P. *et al.* Chronic diffuse infiltrative lung disease: Determination of the diagnostic value of clinical data, chest radiography, and CT with Bayesian analysis. *Radiology* **191**, 383–390 (1994).

84. Drent, M. *et al.* Sarcoidosis: Assessment of disease severity using HRCT. *Eur. Radiol.* **13**, 2462–2471 (2003).

85. Thillai, M. *et al.* BTS Clinical Statement on pulmonary sarcoidosis. *Thorax* **76**, 4–20 (2021).

86. Cremers, J. *et al.* Extent of disease activity assessed by 18F-FDG PET/CT in a Dutch population. *Sarcoidosis Vasc Diffus. Lung Dis* . **31**, 37–45 (2014).

87. Chen, X., Xu, X., Chrysikos, S., Zhao, M. & Zhou, Y. Value of 18-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) in the differential diagnosis of sarcoidosis and lung cancer with lymph node metastasis: a retrospective study. *Transl. Lung Cancer Res.* **11**, 1926–1935 (2022).

88. Kim, S.-J., Pak, K., Kim, K. & Kim, J. Diagnostic performance of F-18 FDG PET for detection of cardiac sarcoidosis; A systematic review and meta-analysis. *J Nucl Cardiol.* **27**, 2103–2115 (2020).

89. Kemal, C. T. *et al.* The importance of PET/CT findings and hematological parameters in prediction of progression in sarcoidosis cases. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **34**, 242–250 (2017).

90. Vorselaars, A. D. *et al.* Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur. Respir. J.* **43**, 602–609 (2014).

91. Adams, H. *et al.* Volumetric FDG PET analysis of global lung inflammation: new tool for precision medicine in pulmonary sarcoidosis? *Sarcoidosis, Vasc. Diffus. Lung Dis.* **35**, 44 (2018).

92. van Moorsel, C. H.M., Pertrek, M. & Rivera, N. V. Unravelling the genetic basis of sarcoidosis. in *Sarcoidosis (ERS Monograph)* 41–56 (2022).

93. 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* **156**, 1601–1605 (1997).

94. Rivera, N. V *et al.* High-Density Genetic Mapping Identifies New Susceptibility Variants in Sarcoidosis Phenotypes and Shows Genomic-driven Phenotypic Differences. *Am. J. Respir. Crit. Care Med.* **193**, 1008–1022 (2016).

95. De Bakker, P. I. W. *et al.* A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat. Genet.* **38**, 1166–1172 (2006).

96. Juge, P.-A. *et al.* MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease . *N. Engl. J. Med.* **379**, 2209–2219 (2018).

97. Ley, B. *et al.* The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis: an observational cohort-control study. *Lancet. Respir. Med.* **5**, 639–647 (2017).

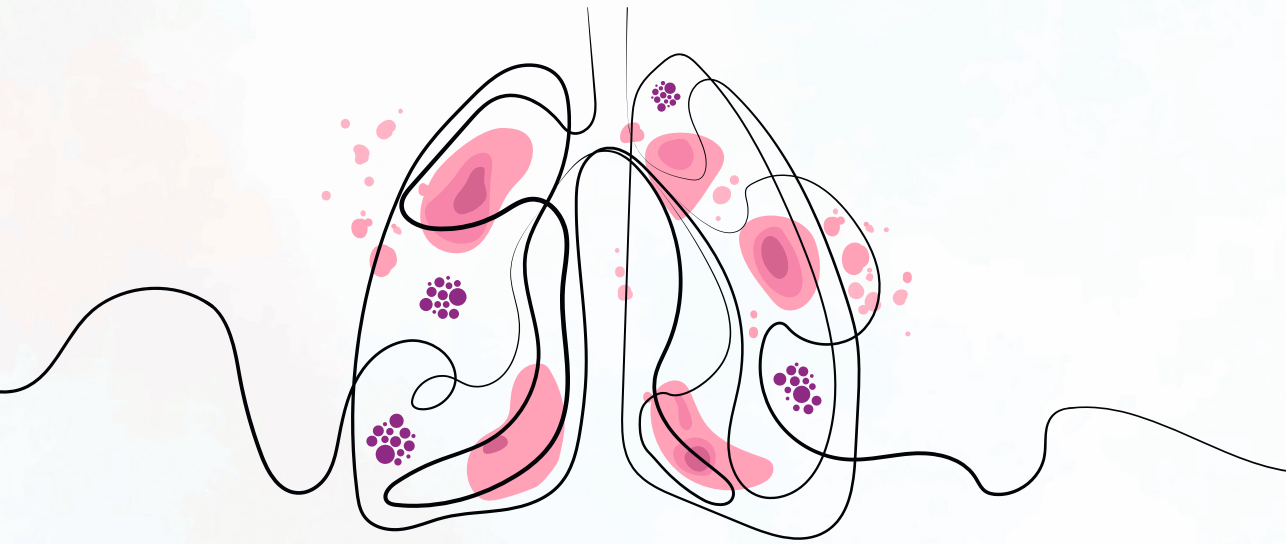
98. Fingerlin, T. E. *et al.* Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat. Genet.* **45**, 613–620 (2013).

99. Seibold, M. A. *et al.* A Common MUC5B Promoter Polymorphism and Pulmonary Fibrosis. *N Engl J Med.* **364**, 1503–1512 (2011).

100. Zhang, Y., Noth, I., Garcia, J. G. N. & Kaminski, N. A variant in the promoter of MUC5B and idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **364**, 1576–1577 (2011).

101. Noth, I. *et al.* Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet. Respir. Med.* **1**, 309–317 (2013).

102. Stock, C. J. *et al.* Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax* **68**, 436–441 (2013).



CHAPTER 2

BIOMARKERS IN SARCOIDOSIS

M.C. Schimmelpennink, A.D.M. Vorselaars, J.C. Grutters

Sarcoidosis: A Clinician's Guide, 2018; Chapter 19; 219-238

ABSTRACT

This chapter presents the most commonly used markers in sarcoidosis: serum angiotensin converting enzyme (ACE), serum soluble interleukin-2 receptor (sIL-2R), serum and urinary calcium, serum lysozyme, bronchoalveolar lavage (BAL) lymphocytosis, BAL CD4+/CD8+ ratio, and BAL CD103+CD4+/CD4+ ratio. The following purposes of biomarkers in sarcoidosis care are discussed: the diagnostic value and measure to assess the burden of the granulomatous inflammatory process and the prognostic value, to determine severity of organ involvement (staging), to evaluate the response of therapy or to predict relapse of disease. Furthermore, potential biomarkers and recent developments in the field of sarcoidosis detection and prognostication are reviewed, such as the use of gene signatures.

SERUM AND BAL BIOMARKERS IN PULMONARY AND EXTRA-PULMONARY SARCOIDOSIS

The definition of a biomarker according to the National Institutes of Health Biomarkers Definitions Working Group is "A characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"[1]. In sarcoidosis care, biomarkers can be useful in many ways: to diagnose sarcoidosis, to predict prognosis and determine severity, to evaluate the response to therapy, or to predict relapse. The ideal biomarker is highly sensitive and is not related to other diseases. Furthermore, it should be reproducible, not very invasive to the patient, and ideally it should be low priced. Unfortunately, none of the currently used biomarkers in sarcoidosis care fulfil all these criteria[2]. The best way to determine the diagnostic value of a biomarker is by determining the area under the receiver operating characteristic (ROC) curve (AUC). An AUC of 0.9–1.0 is considered excellent, 0.80–0.90 is good, 0.70–0.80 is fair, 0.60–0.70 is poor, and 0.50–0.60 is bad.

The following biomarkers for sarcoidosis most commonly used around the world ('conventional biomarkers') will be discussed: serum angiotensin-converting enzyme (sACE), serum soluble interleukin-2 receptor (sIL-2R), serum and urinary calcium, serum lysozyme, bronchoalveolar lavage (BAL) lymphocytosis, and BAL lymphocyte subset parameters (table 1). In addition, we will highlight some recent developments and potential biomarkers for future use.

CONVENTIONAL BIOMARKERS

Serum angiotensin-1-converting enzyme

sACE converts blood angiotensin I into angiotensin II during circulation through the lungs[3]. In sarcoidosis, increased ACE levels is the result of additional production by activated macrophages and epithelioid cells in the granulomas[4–6]. As such, serum levels of ACE in sarcoidosis are thought to reflect the total-body burden of granulomas[7].

Clinical sensitivity of an elevated sACE level for pulmonary sarcoidosis roughly varies between 41% and 83%[8–10], with the following AUCs: 0.656 (poor), 0.643 (poor), and 0.790 (fair) (table 1). Importantly the sensitivity of sACE depends on the clinical manifestations of sarcoidosis. In patients with sarcoid uveitis, the sensitivity of sACE lies between 22% and 54%[11,12], with the following AUCs: 0.650 (poor) and 0.608 (poor). In patients

diagnosed with neurosarcoidosis, the sensitivity of sACE lies around 44%[13]; however, AUC could not be estimated because of the lack of a control group.

Of particular importance is that the sensitivity of sACE has been shown to be affected by a common polymorphism in the ACE gene. This genetic polymorphism is characterised by the insertion (I) or deletion (D) of the 287 base-pair fragment in intron 16. Patients with an II genotype have lower levels of sACE than patients with DD genotypes, and patients with ID genotype have intermediate sACE levels[14]. Using I/D-corrected reference intervals, 8.5% of the measurements lead to a different interpretation[15]. Genotype-corrected levels of sACE show improved sensitivity of 69%–83% for diagnosing sarcoidosis[16,17].

Furthermore, sACE has a limited specificity (table 1). Increased levels of sACE can also be found in other granulomatous diseases, such as tuberculosis, histoplasmosis, and leprosy[18–20].

It is uncertain if sACE reflects the severity of sarcoidosis. Some studies found higher sACE levels and a higher sensitivity of sACE in more advanced radiographic stages of sarcoidosis[5,21,22]. However, other studies did not confirm this correlation[23–26]. No prognostic value of sACE measurement in sarcoidosis has so far been demonstrated. sACE cannot predict the need for therapy[23] and progression of disease[27], nor predict relapse after therapy[28].

However, serial measurements of sACE might provide valuable information on the course of disease in individual patients (table 5). First, it has been shown that change in serial measurement corresponds to the course of disease as well as to clinical response to systemic therapy[5,21,29–33]. Second, during treatment, the magnitude of change in sACE was found to correlate significantly to the change in pulmonary function parameters[30,34]. For this reason, serial measurement of sACE still appears useful in the follow-up of disease and assessment of therapeutic response in individual patients. Of note, sACE levels can be influenced by corticosteroids independently, which should be taken into account when evaluating treatment response during corticosteroid therapy[33]. Also, in patients using ACE inhibitors, measurement of sACE levels is not useful[35].

In summary, diagnostic value of sACE in sarcoidosis is limited (sensitivity/specificity: poor-fair), but has not been properly reevaluated in the context of the D/I gene polymorphism. Furthermore, serial measurement of sACE can be useful in the follow-up of disease, especially in the evaluation of nonsteroid systemic therapy. There is currently no evidence that sACE can serve as a prognostic biomarker and/or marker for staging of severity in sarcoidosis.

Table 1. Serum ACE: sensitivity, specificity, severity biomarker, and prognosis

Study	Inclusion	Subtype	Control group	Cut off Value	Sensitivity	Specificity	Severity	Prognosis	AUC
De Smet 2010[8]	(n=36) Biopsy proven, except for 1 patient with LS, no corticosteroids during diagnostic workup	Pulmonary	(n=117) Patients who were evaluated for sarcoidosis, but sarcoidosis was ruled out	34U/L	83.3%	66.7%			0.79
Popevic 2016[9]	(n=430) Biopsy-proven sarcoidosis patients. Patients with active sarcoidosis[a]		(n=264) Healthy controls matched for age and gender.	32U/L	66%	54%	No differences in sACE levels between different Scadding stages. No correlation between COS[b] categories and sACE		0.643
Sharma 1997[16]	(n=47) White patients, histologically proven sarcoidosis. No use of steroids, diuretics, or ACE inhibitors at the time of evaluation		(n=146) Healthy white volunteers	Not genotype corrected: 15-70U/L Genotype corrected: II: 4.6–30.6U/L ID: 10.0–47.6U/L DD: 17.9–64.3U/L	51.7% 69%				
Tomita 1997[17]	(n=207) Biopsy proven, no corticosteroids during evaluation			Not genotype corrected: 8.3–21.4IU/L Genotype-corrected: II: 9–12.5 IU/L DI: 10.5–17.1IU/L DD: 11.9–22.5IU/L	60.8% 83%		Increase in sACE was seen in more advanced radiographic stages		

Table 1. (Continued)

Study	Inclusion	Subtype	Control group	Cut off Value	Sensitivity	Specificity	Severity	Prognosis	AUC
Groen -Hakan 2017[11]	(n=37) Definitive or presumed ocular sarcoidosis based on the International Workshop on Ocular Sarcoidosis criteria (biopsy or radiologic finding). 36% used immunosuppressive medication and 16% used ACE inhibitor of the whole (sarcoidosis + control) group.	Sarcoid uveitis	(n=212) Patients with other or unknown causes of uveitis, including patients with probable or possible uveitis	51U/L	54%	70%			0.65
Gundlach 2016[12]	(n=42) Patients with intraocular sarcoidosis; definite n=10, presumed n=12, probable n=19, and possible n=1. ACE inhibitors were taken by 2 patients in the sarcoid group and 1 patient in the control group.	Sarcoid uveitis	(n=12) Uveitis without sarcoidosis	82U/mL	22%	99.5%			0.608[c]

Table 1. (Continued)

Study	Inclusion	Subtype	Control group	Cut off Value	Sensitivity	Specificity	Severity	Prognosis	AUC
Ungprasert 2016[10]	(n=251) Histological evidence was required, except for stage I pulmonary sarcoidosis in the absence of other causes. Isolated granulomatous disease of an organ except for skin was also included in the absence of a better alternative. Data concerning use of ACE inhibitors are unknown.		(n = 3277) Residents of Olmsted County who had ACE levels tested but did not have a diagnosis of sarcoidosis.	ACE levels were recorded as high/low/ normal according to the reference range for the time the tests were performed.	41.4%	89.9%			0.656[c]
Leonhard 2016[13]	(n=52) 1 patient with definite, 37 probable, and 14 possible neurosarcoidosis (Zajicek criteria)	Neurosarcoidosis		>70 U/L	44%			Elevated ACE levels were more frequent in patients with neurosarcoidosis and pulmonary sarcoidosis (65%) than patients. with only neurosarcoidosis (1%).	

ACE, angiotensin-converting enzyme; *AUC*, area under the curve; *LS,* Löfgren's syndrome; *D*, deletion; *I*, insertion

aActive sarcoidosis, defined as the presence of clinical symptoms, with/without pathological X-ray, or asymptomatic patients with absolute and marked roentgenographic worsening, or patients with major manifestations such as recently developed skin lesions, parotid/ocular involvement, peripheral lymphadenopathy, or deterioration of pulmonary function tests.

bClinical outcome status (COS)

cArea under the ROC curve was not evaluated in this study; the AUC was estimated by the following formula: (0.5 × [sensitivity × (1−specificity)]) + (0.5 × [specificity × (1−sensitivity)]) + (sensitivity × specificity).

Serum soluble interleukin-2 receptor

Serum soluble interleukin-2 (sIL-2R) is released by activated T lymphocytes and correlates well with CD4+ T lymphocytes in BAL and serum[36] and is therefore regarded as a parameter for activation of T lymphocytes[37]. Although it is mainly produced by T lymphocytes, also small amounts of sIL-2R are produced by B lymphocytes[38]. Serum sIL-2R binds to interleukin-2 and plays an important role in the immune response. Sensitivity of serum sIL-2R as a diagnostic biomarker for sarcoidosis lies around 79% (table 2). However, no studies have been performed to the diagnostic accuracy of serum sIL-2R in patients with (pulmonary) sarcoidosis compared with a control group. Therefore, no ROC curve analysis could so far be performed. In patients with uveitis the sensitivity of elevated sIL-2R levels to establish underlying sarcoidosis is around 81%– 98%[11,12], with an AUC of 0.76 (fair) and 0.96 (excellent). Interestingly, patients with extrapulmonary involvement have been shown to have relatively high levels of serum sIL-2R, suggesting a value as staging and/or severity biomarker[36,39]. Also, increase of serum sIL-2R values appears not to be specific for sarcoidosis as elevated values can also be found in other conditions, including hematologic malignancies, other granulomatous diseases, various autoimmune disorders, and post-transplantation[40–44]. An important pitfall in using sIL-2R as a biomarker in sarcoidosis is that renal insufficiency may have major impact on levels leading to misinterpretation of test results[45,46]. Interestingly, serum sIL-2R levels might be useful as a marker of severity of sarcoidosis. Patients with more advanced radiographic stages and progressive disease show higher levels of sIL-2R[47–49] (Table 2). Rothkrantz-Kos et al[50]. evaluated the diagnostic accuracy of inflammatory markers to predict respiratory severity, defined by diffusing capacity for carbon monoxide (DLCO) <80% of predicted, forced vital capacity (FVC) <80% of predicted, or forced expiratory volume 1 (FEV1) <80% of predicted. Serum sIL-2R test had the highest ability to determine pulmonary severity in comparison to ACE. Serum sIL-2R measurement has also shown usefulness as a prognostic marker. High serum sIL-2R levels can predict the need for therapy in sarcoidosis patients[50,51]. Furthermore, high sIL-2R at initiation of therapy has shown value as a predictor of relapse after therapy with infliximab[28]. Serial measurement of serum sIL-2R in the follow-up can be useful to assess the evolution of disease activity in sarcoidosis (table 5). Changes in concentration of serum sIL-2R have been shown to be related to clinical changes and correlate well with changes in pulmonary function parameters and radiological abnormalities[30,36,52,53] (table 5).

Table 2. Serum sIL-2R: sensitivity, specificity, severity biomarker, and prognosis

Study	Inclusion	Subtype	Control group	Cutoff value	Sensitivity	Specificity	Severity	Prognosis	AUC
Grutters 2003[36]	(n=57) Diagnosis was confirmed by histologic evidence. All patients had active disease. No treatment during evaluation.[a]	Only pulmonary sarcoidosis (n=25); pulmonary and extra-pulmonary disease (n=21); 1 patient with solely extra-pulmonary disease		Normal range given by manufacturer 223–710U/mL	79%		Patients with pulmonary and extrapulmonary sarcoidosis (excluding erythema nodosum) had higher sIL-2R levels than patients with only pulmonary disease. sIL-2R was not associated with change in lung function during follow-up. Lowest sIL-2R was in Scadding stage III and highest in stage I.	Initial sIL-2R could not predict chronic disease on chest radiograph at 2 years.	
Groen-Hakan 2017[11]	(n=37) Definitive or presumed ocular sarcoidosis based on the International Workshop on Ocular Sarcoidosis criteria (biopsy or radiologic finding). 36% used immunosuppressive medication of the whole (sarcoidosis+control) group	Sarcoid uveitis	(n=212) Patients with other causes of uveitis and patients with unknown cause of uveitis, including patients with probable or possible uveitis	4000pg/mL	81%	64%			0.76
Gundlach 2016[12]	(n=42) Patients with intraocular sarcoidosis; definite n=10, presumed n=12, probable n=19, possible n=1.	Sarcoid uveitis	(2=12) Uveitis without sarcoidosis	639U/mL	98%	94%			0.96[c]

Table 2. (Continued)

Study	Inclusion	Subtype	Control group	Cutoff value	Sensitivity	Specificity	Severity	Prognosis	AUC
Rothkrantz-Kos 2003[50]	(n=73) Diagnosis was histologically confirmed. Evaluation of markers was in untreated patients. No comorbidities.[b]						sIL-2R had the highest ability to determine pulmonary severity defined by DLCO <80%, FVC <80%, FEV1 <80% of predicted	73% of the untreated nonchronic patients with high values of sIL-2R required eventually treatment, vs. 23% with low sIL-2R	

AUC, area under the curve; *DLCO*, diffusion capacity for carbon monoxide; *FEV1*, forced expiratory volume 1; *FVC*, forced vital capacity; *sIL-2R*, soluble interleukin-2 receptor. aActive disease was defined as: (1) recently developed or increasing cough or dyspnea; and/or (2) presence of compatible systemic symptoms such as cutaneous lesions, eye manifestations, fever, and athralgia; and/or (3) recently developed abnormalities on chest radiograph; and/or (4) T-lymphocytosis in BAL and/or (5) elevated level of serum angiotensin-converting enzyme (sACE). bSensitivity and specificity to evaluate the diagnostic accuracy of inflammatory markers to predict respiratory severity (RFI) in sarcoidosis. cArea under the ROC curve was not performed in this study, the AUC was estimated by the following formula: (0.5 × [sensitivity × (1−specificity)]) + (0.5 × [specificity × (1−sensitivity)]) + (sensitivity × specificity).

In summary, the value of serum sIL-2R as a diagnostic biomarker for sarcoidosis is not yet fully established. However, the diagnostic value of sIL-2R is fair to excellent in patients presenting with uveitis. Serum sIL-2R measurement might also have prognostic value, especially in the context of infliximab therapy. In contrast to sACE, interpretation of serum sIL-2R levels is not confounded by the use of drugs or immunosuppressants. Finally, serial measurements can be useful in the follow-up of patients and to evaluate treatment effect.

Serum calcium and urinary calcium

Sarcoidosis is associated with an altered calcium metabolism. In sarcoidosis, activated macrophages produce 1α-hydroxylase[54,55]. The enzyme 1α-hydroxylase facilitates the hydroxylation of 25-hydroxyvitamin D (inactive form of vitamin D) to 1,25-dihydroxyvitamin D (calcitriol, the biologically active form of vitamin D). Calcitriol is a sterol hormone that stimulates the intestinal absorption of calcium and bone resorption, which can lead to hypercalcemia and hypercalciuria[56]. Thus, sarcoidosis patients with hypercalcemia and/or hypercalciuria are classically characterised by increased levels of 1,25-dihydroxyvitamin D and decreased levels of 25-hydroxyvitamin. Measurement of serum calcium and urinary calcium belongs to the standard diagnostic workup of sarcoidosis. Hypercalcemia affects only 3.6%–16% of the patients with extrapulmonary and/or pulmonary sarcoidosis[57–61] (table 3); however, when measuring ionised serum calcium, the biologically active form of calcium, the incidence of hypercalcemia lies around the 28%[62] (table 3). Mild-to-moderate elevation of serum calcium is mostly asymptomatic. However, it can cause serious danger when levels rise above 3.0 mmol/L and as such is regarded an absolute indication for treatment[63]. Hypercalcemia and hypercalciuria occur more often in males[60,64] and white patients[65,66]. Hypercalcemia is a rare phenomenon in patients with Löfgren's syndrome[59]. Hypercalciuria in sarcoidosis has a reported prevalence varying from 6.4% to 33% of the patients[58,60–62]. Nephrolithiasis and nephrocalcinosis can be serious consequences of hypercalciuria[67–69]. Moreover, both can lead to renal failure[57,70,71]. Hypercalcemia and hypercalciuria are both markers reflecting granulomatous inflammatory activity in sarcoidosis. Their presence is also associated with a worse prognosis, i.e., these patients are less likely to have spontaneous remission and a higher rate of relapses[61,72] (table 3).

Table 3. Serum calcium and urinary calcium: sensitivity, specificity, severity biomarker, and prognosis

Study	Inclusion	Subtype	Cutoff Value	Prevalence hypercalcemia	Prevalence hypercalciuria	Renal insufficiency/ Nephrocalcinosis	Severity	Prognosis
Baughman 2013[57]	Diagnosis was made according to criteria ATS/ERS/WASOG. 1606 patients were seen during 13576 visits.		10.2 mg/dL	6.6% had hypercalcemia, of whom 0.6% patients with hyperparathyroidism and 6% diagnosis with SAHC (sarcoidosis associated hypercalcemia)		42% of the patients with hypercalcemia had renal insufficiency		
Kamphuis 2014[58]	Diagnosis was made according to criteria of ATS/ERS/WASOG. Serum calcium was measured in 293 patients, and urinary calcium was measured in 89 patients. Serum calcium level was corrected for albumin.		sCa2+>2.65mmol/L and uCa> 5mmol/L	8%	27%		No correlation between serum levels of calcium and sarcoidosis stadium on chest X-ray.	

Table 3. (Continued)

Study	Inclusion	Subtype	Cutoff Value	Prevalence hypercalcemia	Prevalence hypercalciuria	Renal insufficiency/ Nephrocalcinosis	Severity	Prognosis
Hamada 1998[62]	(n=36) In 30 patients, sarcoidosis was histologically proven, and in 6 patients, sarcoidosis was clinically diagnosed. 8 patients received corticosteroids 1>yr before the study. Patients with hyper/ hypoparathyroidism or bone disease were excluded. All patients had normal renal function, except for 2 with histories of nephrolithiasis and slightly reduced creatinine. Calcium was corrected for albumin.		sCa2+>1.26mmol/L sCa2+ adjusted for albumin >2.54mmol/L Hypercalciurea >0.3g/day in males and 0.25 in females	27.8% 5.6%	33.3%	A history of nephrolithiasis was in 8.3% of the patients	sCa2+ >1.23mmol/L suggested extrathoracic involvement with a sensitivity of 50% and a specificity of 100%.	
Darlington 2014[59]	Non-Löfgren's syndrome: n=617. Löfgren's syndrome: n=383. Diagnosis was made according to criteria of WASOG. Incidence of hypercalcemia and/or renal failure was estimated together.	Löfgren's syndrome versus non-Löfgrens syndrome	Hypercalcemia and/or kidney involvement was diagnosed when repeated blood samples with p-calcium >2.60mmol/L and/ or p-creatinine >90umol/L for women and>100umol/L for men.	Non-Löfgren's syndrome: 3.6% Löfgren's syndrome: 0%				

Table 3. (Continued)

Study	Inclusion	Subtype	Cutoff Value	Prevalence hypercalcemia	Prevalence hypercalciuria	Renal insufficiency/ Nephrocalcinosis	Severity	Prognosis
Morimoto 2008[60]	Japanese cohort. All patients had histopathological evidence. Calcium was measured in 842 patients and hypercalciuria in 298 patients.		Unknown	7.4%	6.4%			
Rodriques 2011[72]	(n=137) Diagnosis was based on histological evidence. Abnormal calcium metabolism evaluated.		Unknown	Incidence of abnormal calcium metabolism 20.4%				Relapse was associated with an abnormal calcium metabolism
Doubkova 2015[61]	Sarcoidosis diagnosis was based on ATS/ERS/WASOG criteria. sCa was measured in 301 patients, and urinary calcium was measured in 253 patients.		Normal range serum calcium 2.15–2.55mmol/L Normal range urinary calcium 2.4–7.5mmol/L	16%	33%			Percentage of patients with spontaneous remission was lower in patients with elevated serum calcium than in patients with normal calcium

In conclusion, serum calcium should be measured as part of the diagnostic workup, and hypercalcemia can be an absolute indication to start systemic therapy. Furthermore, both hypercalcemia and hypercalciuria reflect ongoing granulomatous inflammation and can be regarded as biomarkers for disease activity. However, this only applies for a small subset of patients and thus cannot be regarded as useful biomarkers for sarcoidosis in general.

Lysozyme

Lysozyme is a low-weight enzyme with antibacterial activity. This enzyme is found in macrophages and epithelioid cells of granulomas in sarcoidosis[32,73], however not in older lesions[73]. Like with sACE, it has been thought that serum lysozyme levels might reflect the total-body mass of granulomas[74]. Sensitivity of serum lysozyme for the diagnosis of sarcoidosis lies between 69% and 80%[34,75,76], table 4, with the following AUCs: 0.695 (poor) and 0.799 (fair). Elevated serum levels of lysozyme are also found in patients with pulmonary tuberculosis, silicosis, and asbestosis[77–79], limiting the specificity of lysozyme for the diagnosis of sarcoidosis (table 4). Little is known about the value of lysozyme as severity or prognostic biomarker. Serial measurements of lysozyme might, however, be useful to measure response to systemic anti-inflammatory treatment. For example, monitoring of lysozyme levels has been shown to inversely correlate with change of DLCO during follow-up (table 5)[76]. Lysozyme is metabolised in the kidneys; thus in patients with renal impairment, serum lysozyme has to be interpreted with caution[80].

Table 4. Serum lysozyme: sensitivity, specificity, severity biomarker, and prognosis

Study	Inclusion	Subtype	Control Group	Cutoff Value	Sensitivity	Specificity	Severity	Prognosis	AUC
Prior 1990[34]	(n=25) Histologically proven sarcoidosis, all with radiographic evidence of lung involvement	Pulmonary sarcoidosis		Normal range: 0.4 – 1.5mg/L	80%		Patients with higher lysozyme levels had higher radiographic profusion scores and more impaired FVC and DLCO	Pretreatment lysozyme did not correlate with change in lung function and radiographic profusion score after corticosteroids.	
Tomita 1999[76]	(n=110) Sarcoidosis was diagnosed based on clinical picture and the presence of epithelioid cell granulomas in biopsies from the lung, skin, or lymph nodes. All subjects had normal renal function.		(n=30) Patients with other granulomatous disease: summer-type hypersensitivity pneumonitis (n=7), pulmonary tuberculosis (n=20), pulmonary aspergillosis (n = 3).	11.0µg/mL	79%	60%	Maximum lysozyme increases with the number of organs involved. Lysozyme levels were significantly higher in patients with more advanced radiographic stages.		0.695[a]

Table 4. (Continued)

Study	Inclusion	Subtype	Control Group	Cutoff Value	Sensitivity	Specificity	Severity	Prognosis	AUC
Turton 1979[75]	(n=72) Patients with definite sarcoidosis where the clinical diagnosis has been confirmed histologically by tissue biopsy or the Kveim test.		(n=64) with various other diseases affecting the lungs, post-primary pulmonary tuberculosis (n=8), old pulmonary tuberculosis (n=4), cryptogenic fibrosing alveolitis (n=26), asthma (n=8), carcinoma of the bronchus (n=8)	Normal range 0.9-2.6 units	69%	90.7%			0.7985[a]
Leonhard 2016[13]	The whole cohort consisted of 1 patient with definite, 37 probable and 14 possible neurosarcoidosis (Zajicek criteria). Lysozyme was measured in 26 patients.	Neurosarcoidosis		>3.5mg/L	46%				

AUC, area under the curve; *DLCO*, diffusion capacity for carbon monoxide; *FVC*, forced vital capacity.
aArea under the ROC curve was not performed in this study, the AUC was estimated by the following formula: (0.5 × (sensitivity × (1−specificity))) + (0.5 × (specificity × (1−sensitivity))) + (sensitivity × specificity).

Table 5. Serial measurement of biomarkers

Study	Inclusion	Subtype	Duration of Follow-up	Change in Biomarker	Correlation with Course
ACE					
Vorselaars 2015[30]	(n = 114) Sarcoidosis patients started on second-line treatment with methotrexate. sACE was measured before and after methotrexate.	76 patients with pulmonary treatment indication and 38 patients with extrapulmonary treatment indication.	6 months	Mean ACE decreased from 71.4U/L to 54.2U/L	In patients with pulmonary treatment indication sACE correlated with ΔVC, ΔFEV1, and ΔDLCO after methotrexate therapy.
Baughman 1983[33]	(n=36, 55 observations) Diagnosis was biopsy proven in all patients. After first measurement initiation of 40mg/day prednisone. Thereafter, tapering of prednisone.	Pulmonary sarcoidosis	7 weeks of treatment with prednisone, than tapering of prednisone in the following 7-10 months. Mean duration of follow-up of 4.2 months after institution or change in steroid dose.	-	In 6 of 13 patients with clinical deterioration, a rise in ACE was seen. In 10 of 21 patients with clinical improvement, a fall in ACE levels was seen. In patients with no change in clinical scale, 13 patients had a rise and 7 had a fall in ACE. Strong negative relationship in change of steroid dose and change in ACE level.
SIL-2R					
Thi Hong Nguyen 2017[52]	Skin lesions were assessed by skin biopsy. In 44 patients at least two measurements were determined.	Cutaneous sarcoidosis. In 36% of the patients was pulmonary involvement.			Changes in sIL-2R correlated with clinical progress in 90.1%.
Vorselaars 2015[30]	(n = 114) Sarcoidosis patients started on second-line treatment with methotrexate	76 patients with pulmonary treatment indication and 38 patients with an extrapulmonary treatment indication.	6 months	Mean sIL-2R decreased from 4840 pg/ml to 2290 pg/mL	Significant correlation was found between ΔsIL-2R and ΔFVC, ΔDLCO after methotrexate therapy in patients with a pulmonary treatment indication.

Table 5. (Continued)

Study	Inclusion	Subtype	Duration of Follow-up	Change in Biomarker	Correlation with Course
Grutters 2003[36]	(n=14) Diagnosis was confirmed by histologic evidence. All patients had active disease.		2 years		Positive correlation between change in sIL-2R and change in radiographic stage, which remained significant after correction for treatment. The initial sIL-2R level correlated inversely to the extent of change in the follow-up sIL-2R level.
Lawrence 1988[53]	(n=5) Diagnosis was confirmed with biopsy in conjunction with negative histories, cultures, serologic, and cytologic studies for other causes of granuloma formation. All patients were treated for 6 weeks with 40 mg of prednisolone		6 weeks	Mean sIL-2R decreased from 1499U/mL to 476U/mL	All patients showed clinical improvement measured with pulmonary function and chest radiograph, corresponding with a decrease in sIL-2R.
CALCIUM					
Baughman 2013[57]	Diagnosis was made according to criteria of ATS/ ERS/WASOG.				81/86 (94%) patients' hypercalcemia improved, with normalization in 91%. In 9% withdrawal of calcium and vitamin D supplementation normalized the hypercalcemia.

Table 5. (Continued)

Study	Inclusion	Subtype	Duration of Follow-up	Change in Biomarker	Correlation with Course
LYSOZYME					
Prior 1990[34]	(n = 25) Histologically proven sarcoidosis, all with radiographic evidence of lung involvement. Serum lysozyme was measured before treatment and after prednisolone treatment (40 mg daily for 1–2 months followed by gradual tapering to 15 mg daily over the subsequent months).	Pulmonary sarcoidosis	Median duration of 13 months, (range 3–49) months		Fall in lysozyme after corticosteroid treatment correlates with the improvement in DLCO.

Δ, change; *ACE*, angiotensin converting enzyme; *DLCO*, diffusion capacity for carbon monoxide; *FEV1*, forced expiratory volume 1; *FVC*, forced vital capacity; *sIL-2R*, soluble interleukin-2 receptor.

BAL fluid characteristics

In many clinics, BAL is routinely performed in the diagnosis of sarcoidosis[81]. The disease is characterised by an increased percentage of lymphocytes, increased CD4+/CD8+ ratio, and a decreased CD103+CD4+/CD4+ ratio in BAL fluid. Of general importance is the notion that BAL fluid cell counts can be influenced by smoking[82]. Smoking can even mask the typical BAL fluid characteristics of sarcoidosis patients.

Percentage of lymphocytes

Various studies on sensitivity of BAL fluid lymphocytosis have shown percentages varying from 71% to 85%[8,83,84]. And, specificity of this cellular characteristic in BAL has been reported to lay between 68% and 93%[8,83–85] (table 6). ROC curve analysis varied between 0.695 and 0.775 (poor to fair). This is at least in part related to the fact that increased lymphocytes in BAL fluid can also been found in patients with infections, malignancies[86], and in other interstitial lung diseases such as hypersensitivity pneumonitis[87], and in cryptogenic organizing pneumonitis[88]. In literature, there is no consensus if this marker reflects the severity of sarcoidosis. There are reports on higher percentage of lymphocytes in patients with acute presentation of disease[89], which is associated with a favourable course of disease[90]. However, in other studies, no such correlation was found[49,82]. Furthermore, the percentage of lymphocytes in BAL fluid is also found not to be predictive for disease outcome in sarcoidosis[91,92]. In summary, percentage of lymphocytes in BAL fluid can be used as a diagnostic marker for sarcoidosis. However, its test performance has to be qualified as poor to fair. Furthermore, lymphocytosis is not informative in terms of disease severity and/or has no predictive value.

CD4+/CD8+ ratio

Determining CD4+/CD8+ ratio can be helpful in the diagnosis of sarcoidosis. Sarcoidosis is characterised by an increased CD4+/CD8+ ratio (>3.5), compared with other interstitial lung diseases. Sensitivity of the CD4+/CD8+ ratio lies between 54% and 80%[8,83–86,93–95], whereas the specificity varies from 59% to 80%[8,83–86,93,94] (table 6). The area under the ROC curve for CD4+/CD8+ ratio for diagnosing sarcoidosis varies between 0.705 and 0.936, which means that this is a fair-excellent biomarker for diagnosing sarcoidosis.

Table 6. BAL biomarkers lymphocytes (%), CD4+/CD8+ ratio, CD103+CD4+/CD4+ ratio: sensitivity, specificity, and severity biomarker

Study	Inclusion	Subtype	Control group	Cutoff value	Sensitivity	Specificity	Severity	AUC
% LYMPHOCYTES								
Tanriverdi 2016[83]	(n = 68) Diagnosis was confirmed by biopsy. None of the patients received steroids before BAL.	Sarcoidosis patients with diffuse parenchymal lung disease	(n=72) Non-sarcoidosis patients with diffuse parenchymal lung diseases: CTD-ILD (n = 20), pneumoconiosis (n=14), IPF (n = 12), infections (n = 5), other ILD (n = 16), malignancy (n = 4).	9.40%	85%	72%		0.776
De Smet 2010[8]	(n = 36) All biopsy proven, except 1 patient with LS. None of the patients received corticosteroids during diagnostic workup.	Pulmonary sarcoidosis	(n=117) Patients with clinical suspicion of pulmonary sarcoidosis but who turned out to have another diagnosis	18%	75%	74%		0.77
Hyldgaard 2012[84]	(n =17) All patients had biopsy confirmed sarcoidosis.		(n=73) Patients with other pulmonary diseases (EAA, IPF, NSIP, DIP, LIP ILD associated with collagen vascular disease, unclassified interstitial lung disease, TBC, aspergillosis, and other nongranulomatous lung disease)	13%	71%	68%		0.695[a]
CD4+/CD8+ RATIO								
Tanriverdi 2016[83]	(n = 68) Diagnosis was confirmed by biopsy. None of the patients received steroids before BAL.	Sarcoidosis patients with diffuse parenchymal lung disease	(n=72) Nonsarcoidosis patients with diffuse parenchymal lung diseases (DPLD): CTD-ILD, pneumoconiosis, IPF, infections, PAP, COP, NSIP, malignancy.	1.34	76%	79%		0.844
De Smet 2010[8]	(n = 36) All biopsy proven, except 1 patient with LS. None of the patients received corticosteroids during diagnostic workup.	Pulmonary sarcoidosis	(n = 117) Patients with clinical suspicion of pulmonary sarcoidosis but who turned out to have another diagnosis.	2.62	67%	82%		0.79
Suchankova 2013[85]	(n = 26) Sarcoidosis diagnosis was based on ATS/ERS/ WASOG criteria. No subjects received corticosteroids before BAL.	Pulmonary sarcoidosis	(n = 27): IPF (n = 12); CTD-related ILD (n = 7); drug-induced ILD (n = 3); NSIP (n = 3); and radiation-induced ILD (n = 12).	3.5	68%	94%		0.81[a]

Table 6. (Continued)

Study	Inclusion	Subtype	Control group	Cutoff value	Sensitivity	Specificity	Severity	AUC
Heron 2008[93]	(n=119) Sarcoidosis diagnosis was based on ATS/ERS/ WASOG criteria. No subjects received corticosteroids before BAL.	Pulmonary sarcoidosis	(n = 63) other ILD: HP (n = 22); IPF (n = 8); other interstitial pneumonia (n = 3); infection (n = 13); systemic disease (n = 8); malignancy (n = 6); other (n = 3)	2.5	73%	67%		0.81
Danila 2009[94]	(n=318) Diagnosis was confirmed according to ATS/ ERS/ WASOG. Diagnosis was biopsy proven in 98 patients, in other patients, final diagnosis was based on typical picture and radiographic symptoms (e.g., Löfgren's syndrome).	Pulmonary sarcoidosis	(n=185): 55 healthy controls and 130 patients with other disorders who underwent BAL.	3.5	80%	90%	Sensitivity of CD4+/CD8+ ratio decreases in higher radiological stages. Optimal cutoff points for CD4+/CD8+ ratio is 3.5 in asymptomatic and 4.0 in symptomatic patients.	0.936
Hyldgaard 2012[84]	(n=19) All patients had biopsy confirmed sarcoidosis.		(n= 83) Patients with other pulmonary diseases (EAA, IPF, NSIP, DIP,LIP ILD associated with collagen vascular disease, unclassified interstitial lung disease, TBC, aspergillosis, and other nongranulomatous lung disease)	3.8	68%	73%		0.705[a]

Table 6. (Continued)

Study	Inclusion	Subtype	Control group	Cutoff value	Sensitivity	Specificity	Severity	AUC
Winterbauer 1993[86]	(n=27) Diagnostic criteria were a compatible clinical picture, demonstration of multiple noncaseating granuloma or a positive Kveim test, and exclusion of other diseases capable of mimicking sarcoidosis. All patients had>16% lymphocytes. None of the patients had received corticosteroids.		Nonsarcoidosis ILD (n = 28): IPF n = 10, drug-induced pneumonitis n = 7, collagen vascular disease n = 4, radiation pneumonitis n = 4, berylliosis n = 1, BOOP n = 1.	4	59%	96%		0.775[a]
CD103+CD4+/CD4+								
Heron 2008[93]	(n = 119) Diagnosis was based on ATS/ERS/WASOG criteria. No subjects received corticosteroids prior to BAL. Only patients with alveolar lymphocytosis, defined by ≥ 10% lymphocytes in BAL, were included.	Pulmonary sarcoidosis	(n = 63) other ILD: HP (n = 22); IPF (n = 8); other interstitial pneumonia (n = 3); infection (n = 13); systemic disease (n = 8); malignancy (n = 6); other (n = 3)					0.79
Hyldgaard 2012[84]	(n = 19) All patients had biopsy confirmed sarcoidosis.		(n= 83) Patients with other pulmonary diseases (EAA, IPF, NSIP, DIP,LIP ILD associated with collagen vascular disease, unclassified interstitial lung disease, TBC, aspergillosis, and other nongranulomatous lung disease)	<0.22	63%	76%	No correlation between CD103+CD4+/ CD4+ and radiographic staging	0.695[a]
Mota 2012[97]	(n = 41) Diagnosis was confirmed according to ATS/ ERS/WASOG.		(n = 45) Other ILD: HP, IPF, NSIP, COP, SLE, RA, scleroderma, drug-induced lung disease, and silicosis.	0.45	81%	78%		0.86

AUC, area under the curve; *LS*, Löfgren's syndrome; *ILD*, interstitial lung disease; *IPF*, idiopathic pulmonary fibrosis; *SLE*, systemic lupus erythematosus; *RA*, rheumatoid arthritis; HP, hypersensitivity pneumonia; *BAL*, bronchoalveolar lavage; *NSIP*, nonspecific interstital pneumonia; *DIP*, desquamative interstitial pneumonia; *LIP*, lymphoid interstitial pneumonia; *COP*, cryptogenic organizing pneumonia; *EAA*, extrinsic allergic alveolitis; *TBC*, tuberculosis; *BOOP*, bronchiolitis obliterans with organizing pneumonia; *PAP*, pulmonary alveolar proteinosis; *CTD*, connective tissue disease.aArea under the ROC curve was not performed in this study, the AUC was estimated by the following formula: (0.5 × (sensitivity × (1−specificity))) + (0.5 × (specificity × (1−sensitivity))) + (sensitivity × specificity).

The ratio appears to have value as an indicator for the severity of the disease/extend of organ involvement. Some studies found higher CD4+/CD8+ ratio and higher sensitivity of CD4+/CD8+ ratio in less advanced Scadding stages[90,94]. Furthermore, a higher CD4+/CD8+ ratio is found in patients who carry *HLA-DRB1**03, a genotype that is correlated with favourable outcome in sarcoidosis[96]. In addition, many studies have reported relatively high CD4+/CD8+ ratio in patients with acute onset sarcoidosis. This clinical phenotype usually has a favourable prognosis[61,82,89,90,96]. Therefore, measurement of CD4+/CD8+ ratio in BAL fluid also appears to have some prognostic value. In summary, CD4+/CD8+ ratio can be classified as a fair-to-excellent diagnostic marker for sarcoidosis. Furthermore, it has an association with disease severity and outcome, although this has limited clinical value.

CD103+CD4+/CD4+ ratio

Typically in sarcoidosis a decreased CD103+CD4+/CD4+ ratio (<0.2) in BAL fluid is found[93]. Such a decreased CD103+CD4+/CD4+ ratio has a sensitivity of circa 63%–81% and a specificity of 76%–78%[84,97], with an AUC of 0.695 and 0.790 (poor to fair) (table 6). Interestingly, it has been shown that the combined use of the CD4+/CD8+ ratio and the CD103+CD4+/CD4+ ratio increases the specificity for diagnosing sarcoidosis to 91%[93]. It is thought that CD103 positive cells are involved in fibrogenic inflammation[98]. By this means, in more advanced radiologic stages of sarcoidosis, a higher proportion of CD4+ T lymphocytes express CD103[93,97]. Indicating that patients with relatively high CD103+CD4+/CD4+ ratio might have a worse prognosis. In summary, especially the combination of CD4+/CD8+ ratio and CD103+CD4+/CD4+ ratio can be regarded as a specific biomarker for diagnosis of sarcoidosis. CD103+CD4+/CD4+ ratio might be a severity and prognostic biomarker, but this will need further research.

POTENTIAL BIOMARKERS

Chitotriosidase

Chitotriosidase is an enzyme produced by activated macrophages, which plays a role in the defence against fungi, insects, and nematodes[99]. Chitotriosidase can determine disease activity in sarcoidosis with a sensitivity that lies between 89% and 100%[100–102] and a specificity of circa 93%[100]. Chitotriosidase also appears to be an indicator of the severity of sarcoidosis[47,100,102]. Patients with Löfgren's syndrome and with stable disease have lower levels of chitotriosidase[100,103], whereas patients with persistent disease on steroids have the highest levels[100]. Serial measurements of chitotriosidase seem to correlate well with the clinical course of the disease[101,103]. However, like in sACE, it is

possible that treatment with corticosteroids can lower chitotriosidase levels irrespective of the lowering of disease activity[101,102].

Th17 cells and Tregs

Recently, increased amounts of Th17 (CCR4+/CXCR3-) CD4+ T cells have been found in the BAL fluid and the peripheral blood of sarcoidosis patients[104,105]. Th17 CD4+ T cells play a role in many inflammatory diseases. Interestingly, higher BAL fluid Th17.1 cell proportions (i.e., interferon-γ–producing Th17 cells) have been described in patients developing chronic disease than in patients with resolving disease, indicating a potential cellular biomarker to predict disease course in sarcoidosis[106]. Paradoxically, higher frequency of Th17.1 cells has also been observed in Löfgren's syndrome, which usually has a favourable outcome[107].

Another T cell subset, regulatory T cells (Tregs), has been found increased in blood of sarcoidosis patients, most prominently in those developing chronic disease.[108] However, before such potential cellular biomarkers can be acceptable for clinical use, further research is required.

Serum amyloid A

Serum amyloid A (SAA) is a family of proteins produced in the liver which are elevated in an acute-phase response. SAA3 is a member of this family that can be produced extrahepatically in inflammatory tissue by macrophages[109]. SAA depositions are found in the granulomas in sarcoidosis patients[110]. Elevated levels of serum SAA have been found in sarcoidosis[110,111], and higher levels were shown in patients with more active disease[39]. Furthermore, data exist showing that serum SAA levels correlate with severity of lung function impairment and need for systemic therapy[111].

Chemokines

Chemokines are a family of chemoattractant proteins, and certain chemokine actions can stimulate the migration of T cells to inflammatory sites[112]. Increased amounts of IFN-γ–inducible chemokines CXCL9, 10, and 11 and the receptor CXCR3 have been found in the BAL and serum of patients with sarcoidosis[113–118]. As CXCL9 and CXC10 are involved in the migration of Th1 lymphocytes, they might potentially be useful as biomarkers for disease severity and prognosis. Interestingly, CXCL9 and CXCL10 were shown to be inversely correlated with the FVC% and DLCO% of predicted[119]. Furthermore, blood transcriptomic signature reflecting CXCL9 can predict disease outcome longitudinally[120].

FUTURE PURPOSES: OMICS

To identify novel biomarkers, new techniques have been used applying omic technologies, which means profiling of sets of molecules[121]. Gene expression analysis on tissue of sarcoidosis patients mostly reveals overexpression of genes engaging T-helper one response[122]. Especially MMP-12 and ADAMDEC1 transcripts were highly expressed in lung tissue. MMP-12 plays a role in lung remodelling and lung fibrosis; thus this marker can possibly be interesting as a prognostic marker for fibrosing phenotypes of sarcoidosis[122]. One study identified a blood gene signature to diagnose sarcoidosis with 92% sensitivity and 92% specificity[123]. Other studies found that gene expression signatures in biopsies[122,124] and blood[125] of sarcoidosis patients were able to identify patients with a self-limiting disease from patients with more complicated sarcoidosis, also suggesting potential prognostic biomarkers.

Furthermore, proteomic profiling is an interesting development that might reveal new biomarkers in sarcoidosis. A recent study used microarrays built on protein fragments to detect sarcoidosis-associated antigens. This study found four proteins as potential sarcoidosis-associated autoimmune targets[126]. Another study performed a breath analysis by applying electronic nose technology. Interestingly, in the acquired breath prints containing exhaled molecular profiles, untreated pulmonary sarcoidosis patients could be differentiated from healthy controls[127].

CONCLUSION

Commonly used markers in sarcoidosis are serum ACE, sIL-2R, calcium, lysozyme, and urine calcium and cellular parameters in BAL. None of them actually would qualify as an adequate diagnostic, prognostic, and/or staging biomarker. However, still the (combined) use of these markers can be helpful in the appropriate clinical context. A promising horizon is recent publications on gene expression/protein signatures in sarcoidosis. This technology can provide new insights into this enigmatic disease and can help the development of novel biomarkers.

REFERENCES

1. Atkinson, A. J. *et al.* Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **69**, 89–95 (2001).
2. Costabel, U. *et al.* Consensus conference: Activity of sarcoidosis. *Eur. Respir. J.* **7**, 624–627 (1994).
3. Ryan, J. W., Smith, U. & Niemeyer, R. S. Angiotensin I: Metabolism by Plasma Membrane of Lung. *Science (80-.).* **176**, 64–66 (1972).
4. Okabe, T., Suzuki, A., Ishikawa, H., Yotsumoto, H. & Ohsawa, N. Cells originating from sarcoid granulomas in vitro. *Am. Rev. Respir. Dis.* **124**, 608–612 (1981).
5. Lieberman, J. The specificity and nature of serum-angiotensin-converting enzyme (serum ACE) elevations in sarcoidosis. *Ann. N. Y. Acad. Sci.* **278**, 488–497 (1976).
6. Silverstein, E., Pertschuk, L. P. & Friedland, J. Immunofluorescent localization of angiotensin converting enzyme in epithelioid and giant cells of sarcoidosis granulomas. *Proc. Natl. Acad. Sci. U. S. A.* **76**, 6646–6648 (1979).
7. Muthuswamy, PP, Lopez Majano, V, Ranginwala, M, Trainor, W. Serum angiotensin-converting enzyme (SACE) activity as an indicator of total body granuloma load and prognosis in sarcoidosis. *Sarcoidosis* **4**, 142–8 (1987).
8. De Smet, D. *et al.* Use of likelihood ratios improves interpretation of laboratory testing for pulmonary sarcoidosis. *Am. J. Clin. Pathol.* **134**, 939–947 (2010).
9. Popevic, S. *et al.* Verifying Sarcoidosis Activity: Chitotriosidase versus ACE in Sarcoidosis - a Case-control Study. *J. Med. Biochem.* **35**, 390–400 (2016).
10. Ungprasert, P., Carmona, E. M., Crowson, C. S. & Matteson, E. L. Diagnostic Utility of Angiotensin-Converting Enzyme in Sarcoidosis: A Population-Based Study. *Lung* **194**, 91–95 (2016).
11. Groen-Hakan, F. *et al.* Diagnostic value of serum-soluble interleukin 2 receptor levels vs angiotensin-converting enzyme in patients with sarcoidosis-associated uveitis. *JAMA Ophthalmol.* **135**, 1352–1358 (2017).
12. Gundlach, E., Hoffmann, M. M., Prasse, A., Heinzelmann, S. & Ness, T. Interleukin-2 Receptor and Angiotensin-Converting Enzyme as Markers for Ocular Sarcoidosis. *PLoS One* **11**, (2016).
13. Leonhard, S. E. *et al.* Neurosarcoidosis in a Tertiary Referral Center: A Cross-Sectional Cohort Study. *Medicine (Baltimore).* **95**, (2016).
14. Rigat, B. *et al.* An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest.* **86**, 1343–1346 (1990).
15. Kruit, A. *et al.* ACE I/D-corrected Z-scores to identify normal and elevated ACE activity in sarcoidosis. *Respir. Med.* **101**, 510–515 (2007).
16. Sharma, P. *et al.* Clinical value of ACE genotyping in diagnosis of sarcoidosis. *Lancet (London, England)* **349**, 1602–1603 (1997).
17. Tomita, H. *et al.* Polymorphism in the angiotensin-converting enzyme (ACE) gene and sarcoidosis. *Am. J. Respir. Crit. Care Med.* **156**, 255–259 (1997).

18. Brice, E. A. W., Friedlander, W., Bateman, E. D. & Kirsch, R. E. Serum angiotensin-converting enzyme activity, concentration, and specific activity in granulomatous interstitial lung disease, tuberculosis, and COPD. *Chest* **107**, 706–710 (1995).

19. Ryder, K. W., Jay, S. J., Kiblawi, S. O. & Hull, M. T. Serum Angiotensin Converting Enzyme Activity in Patients With Histoplasmosis. *JAMA J. Am. Med. Assoc.* **249**, 1888–1889 (1983).

20. Lieberman, J. & Rea, T. H. Serum angiotensin-converting enzyme in leprosy and coccidioidomycosis. *Ann. Intern. Med.* **87**, 422–425 (1977).

21. Rohrbach, M. S. & Deremee, R. A. Serum angiotensin converting enzyme activity in sarcoidosis as measured by a simple radiochemical assay. *Am. Rev. Respir. Dis.* **119**, 761–767 (1979).

22. Rohatgi, P. K. & Ryan, J. W. Simple radioassay for measuring serum activity of angiotensin-converting enzyme in sarcoidosis. *Chest* **78**, 69–76 (1980).

23. Lieberman, J., Schleissner, L. A., Nosal, A., Sastre, A. & Mishkin, F. Clinical correlations of serum angiotensin-converting enzyme (ACE) in sarcoidosis. A longitudinal study of serum ACE, 67gallium scans, chest roentgenograms, and pulmonary function. *Chest* **84**, 522–528 (1983).

24. Bunting, P., Szalai, J. & Katic, M. Diagnostic aspects of angiotensin converting enzyme in pulmonary sarcoidosis. *Clin Biochem* **20**, 213–9 (1987).

25. Studdy, P., Bird, R. & James, D. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. *Lancet* **2**, 1331–1334 (1978).

26. Specks, U., Martin, W. J. & Rohrbach, M. S. Bronchoalveolar lavage fluid angiotensin-converting enzyme in interstitial lung diseases. *Am. Rev. Respir. Dis.* **141**, 117–123 (1990).

27. Ziegenhagen, M. W. *et al.* Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am. J. Respir. Crit. Care Med.* **156**, 1586–1592 (1997).

28. Vorselaars, A. D. *et al.* Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur. Respir. J.* **43**, 602–609 (2014).

29. Klech, H., Kohn, H., Kummer, F. & Mostbeck, A. Assessment of activity in Sarcoidosis. Sensitivity and specificity of 67Gallium scintigraphy, serum ACE levels, chest roentgenography, and blood lymphocyte subpopulations. *Chest* **82**, 732–738 (1982).

30. Vorselaars, A. D. *et al.* ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. *Respir. Med.* **109**, 279–285 (2015).

31. Vorselaars, A. D. *et al.* Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur. Respir. J.* **46**, 175–185 (2015).

32. Silverstein, E., Friedland, J. & Ackerman, T. Elevation of granulomatous lymph-node and serum lysozyme in sarcoidosis and correlation with angiotensin-converting enzyme. *Am. J. Clin. Pathol.* **68**, 219–224 (1977).

33. Baughman, R. P., Ploysongsang, Y., Roberts, R. D. & Srivastava, L. Effects of sarcoid and steroids on angiotensin-converting enzyme. *Am. Rev. Respir. Dis.* **128**, 631–633 (1983).

34. Prior C, Barbee, RA, Evans, P. Lavage versus serum measurements of lysozyme, angiotensin converting enzyme and other inflammatory markers in pulmonary sarcoidosis. *Eur Respir J.* **3**, 1146–1154 (1990).

35. Krasowski, M. D. *et al.* Ordering of the Serum Angiotensin-Converting Enzyme Test in Patients Receiving Angiotensin-Converting Enzyme Inhibitor Therapy: An Avoidable but Common Error. *Chest* **148**, 1447–1453 (2015).

36. Grutters, J. C. *et al.* Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest* **124**, 186–195 (2003).

37. Rubin, L. A. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol. (Baltimore, Md. 1950)* **135**, 3172–3177 (1985).

38. Nelson, D. L., Rubin, L. A., Kurman, C. C., Fritz, M. E. & Boutin, B. An analysis of the cellular requirements for the production of soluble interleukin-2 receptors in vitro. *J. Clin. Immunol.* **6**, 114–120 (1986).

39. Gungor, S. *et al.* Conventional markers in determination of activity of sarcoidosis. *Int. Immunopharmacol.* **25**, 174–179 (2015).

40. Rubin, L. A. & Nelson, D. L. The soluble interleukin-2 receptor: Biology, function, and clinical application. *Ann. Intern. Med.* **113**, 619–627 (1990).

41. Greene, W. C., Leonard, W. J., Depper, J. M., Nelson, D. L. & Waldmann, T. A. The human interleukin-2 receptor: normal and abnormal expression in T cells and in leukemias induced by the human T-lymphotropic retroviruses. *Ann. Intern. Med.* **105**, 560–572 (1986).

42. Brown, A. E., Rieder, K. T. & Webster, H. K. Prolonged elevations of soluble interleukin-2 receptors in tuberculosis. *Am. Rev. Respir. Dis.* **139**, 1036–1038 (1989).

43. Tung, KS, Umland E, Matzner P, N. K. Soluble serum interleukin 2 receptor levels in leprosy patients. 1987; *Clin Exp Immunol.* **69**, 10–15 (1987).

44. Wolf, R. E. & Brelsford, W. G. Soluble interleukin-2 receptors in systemic lupus erythematosus. *Arthritis Rheum.* **31**, 729–735 (1988).

45. Verwoerd, A. *et al.* Discrepant elevation of sIL-2R levels in sarcoidosis patients with renal insufficiency. *Eur. Respir. J.* **46**, 277–280 (2015).

46. Takamatsu T, Yasuda N, Ohno T, Kanoh T, Uchino H, F. A. Soluble interleukin-2 receptors in the serum of patients with chronic renal failure. *Tohoku J Exp Med.* **155**, 343–347 (1988).

47. Bargagli, E. *et al.* Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. *Scand. J. Clin. Lab. Invest.* **68**, 479–483 (2008).

48. Ina, Y. *et al.* Soluble interleukin 2 receptors in patients with sarcoidosis. Possible origin. *Chest* **102**, 1128–1133 (1992).

49. Ziegenhagen, M. W., Rothe, M. E., Schlaak, M. & Muller-Quernheim, J. Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. *Eur. Respir. J.* **21**, 407–413 (2003).

50. Rothkrantz-Kos, S., van Dieijen-Visser, M. P., Mulder, P. G. & Drent, M. Potential usefulness of inflammatory markers to monitor respiratory functional impairment in sarcoidosis. *Clin. Chem.* **49**, 1510–1517 (2003).

51. Prasse, A. *et al.* Phenotyping sarcoidosis from a pulmonary perspective. *Am. J. Respir. Crit. Care Med.* **177**, 330–336 (2008).

52. Thi Hong Nguyen, C., Kambe, N., Kishimoto, I., Ueda-Hayakawa, I. & Okamoto, H. Serum soluble interleukin-2 receptor level is more sensitive than angiotensin-converting enzyme or lysozyme for diagnosis of sarcoidosis and may be a marker of multiple organ involvement. *J. Dermatol.* **44**, 789–797 (2017).

53. Lawrence, E. C. *et al.* Elevated concentrations of soluble interleukin-2 receptors in serum samples and bronchoalveolar lavage fluids in active sarcoidosis. *Am. Rev. Respir. Dis.* **137**, 759–764 (1988).

54. Adams, J. S. *et al.* Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar macrophages in sarcoidosis. *J. Clin. Endocrinol. Metab.* **60**, 960–966 (1985).

55. Bell, N. H., Stern, P. H., Pantzer, E., Sinha, T. K. & DeLuca, H. F. Evidence that increased circulating 1 alpha, 25-dihydroxyvitamin D is the probable cause for abnormal calcium metabolism in sarcoidosis. *J. Clin. Invest.* **64**, 218–225 (1979).

56. Jones, G., Strugnell, S. A. & DeLuca, H. F. Current understanding of the molecular actions of vitamin D. *Physiol. Rev.* **78**, 1193–1231 (1998).

57. Baughman, Robert P., Janovcik, J, Ray, M, Sweiss, N, Lower, E. Calcium and vitamin D metabolism in sarcoidosis. *Sarcoidosis Vasc Diffus. Lung Dis.* **30**, 113–20 (2013).

58. Kamphuis, L. S., Bonte-Mineur, F., Laar van, J. A., Hagen van, P. M. & Daele van, P. L. Calcium and vitamin D in sarcoidosis: is supplementation safe? *J. Bone Miner. Res.* **29**, 2498–2503 (2014).

59. Darlington, P. *et al.* HLA-alleles associated with increased risk for extra-pulmonary involvement in sarcoidosis. *Tissue Antigens* **83**, 267–272 (2014).

60. Morimoto, T. *et al.* Epidemiology of sarcoidosis in Japan. *Eur Respir J.* **31**, 372–9 (2008).

61. Doubková, M., Pospíšil, Z., Skřičková, J. & Doubek, M. Prognostic markers of sarcoidosis: An analysis of patients from everyday pneumological practice. *Clin. Respir. J.* **9**, 443–449 (2015).

62. Hamada, K., Nagai, S., Tsutsumi, T. & Izumi, T. Ionized calcium and 1,25-dihydroxyvitamin D concentration in serum of patients with sarcoidosis. *Eur. Respir. J.* **11**, 1015–1020 (1998).

63. Bilezikian, J. P. Management of acute hypercalcemia. *N. Engl. J. Med.* **326**, 1196–1203 (1992).

64. Zurkova, M., Kolek, V., Tomankova, T. & Kriegova, E. Extrapulmonary involvement in patients with sarcoidosis and comparison of routine laboratory and clinical data to pulmonary involvement. *Biomed. Pap. Med. Fac. Univ. Palacky. Olomouc. Czech. Repub.* **158**, 613–620 (2014).

65. Judson, M. A., Boan, A. D. & Lackland, D. T. The clinical course of sarcoidosis: presentation, diagnosis, and treatment in a large white and black cohort in the United States. *Sarcoidosis, Vasc. Diffus. Lung Dis. Off. J. WASOG* **29**, 119–127 (2012).

66. Baughman, R. P. *et al.* Clinical Characteristics of Patients in a Case Control Study of Sarcoidosis. *Am J Respir Crit Care Med* **164**, 1885–1889 (2001).

67. Rizzato, G, Colombo, P. Nephrolithiasis as a presenting feature of chronic sarcoidosis: a prospective study. *Sarcoidosis Vasc Diffus. Lung Dis.* **13**, 167–72 (1996).

68. Capolongo, G. *et al.* Vitamin-D status and mineral metabolism in two ethnic populations with sarcoidosis. *J. Investig. Med.* **64**, 1025–1034 (2016).

69. Lebacq, E., Verhaegen, H. & Desmet, V. Renal involvement in sarcoidosis. *Postgrad. Med. J.* **46**, 526–529 (1970).

70. Loffler, C. *et al.* Renal sarcoidosis: epidemiological and follow-up data in a cohort of 27 patients - PubMed. *Sarcoidosis Vasc Diffus. Lung Dis* . **31**, 306–315 (2015).

71. Mahfoudhi, M., Mamlouk, H., Turki, S. & Kheder, A. Systemic sarcoidosis complicated of acute renal failure: about 12 cases. *Pan Afr. Med. J.* **22**, (2015).

72. Rodrigues, S. *et al.* Factor analysis of sarcoidosis phenotypes at two referral centers in Brazil - PubMed. *Sarcoidosis Vasc Diffus. Lung Dis.* **28**, 34–43 (2011).

73. Selroos, O. B. N. Biochemical markers in sarcoidosis. *Crit. Rev. Clin. Lab. Sci.* **24**, 185–216 (1986).

74. Selroos, Olof B.N., Klockars, M. Serum lysozyme in sarcoidosis. Evaluation of its usefulness in determination of disease activity - PubMed. *Scand J Respir Dis.* **58**, 110–116 (1977).

75. Turton, C. W. G. *et al.* Value of measuring serum angiotensin I converting enzyme and serum lysozyme in the management of sarcoidosis. *Thorax* **34**, 57–62 (1979).

76. Tomita, H. *et al.* Serum lysozyme levels and clinical features of sarcoidosis. *Lung* **177**, 161–167 (1999).

77. Gronhagen-Riska, C., Kurppa, K., Fyhrquist, F. & Selroos, O. Angiotensin-converting enzyme and lysozyme in silicosis and asbestosis. *Scand. J. Respir. Dis.* **59**, 228–231 (1978).

78. Koskinen, H., Nordman, H. & Froseth, B. Serum lysozyme concentration in silicosis patients and workers exposed to silica dust. *Eur. J. Respir. Dis.* **65**, 481–485 (1984).

79. Perillie, P. E., Khan, K. & Finch, S. C. Serum lysozyme in pulmonary tuberculosis. *Am. J. Med. Sci.* **265**, 297–302 (1973).

80. Pruzanski, W. & Platts, M. E. Serum and urinary proteins, lysozyme (muramidase), and renal dysfunction in mono- and myelomonocytic leukemia. *J. Clin. Invest.* **49**, 1694–1708 (1970).

81. 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 ER. *Am. J. Respir. Crit. Care Med.* **160**, 736–755 (1999).

82. Drent, M., Van Velzen-Blad, H., Diamant, M., Hoogsteden, H. C. & Van den Bosch, J. M. M. Relationship between presentation of sarcoidosis and T lymphocyte profile; A study in bronchoalveolar lavage fluid. *Chest* **104**, 795–800 (1993).

83. Tanrıverdi, H, Uygur, F, Örnek, T, Erboy, F, Altınsoy, B, Atalay, F, Büyükuysal, M, Tekin, I, Arasli, M, Tor, M. Comparison of the diagnostic value of different lymphocyte subpopulations in bronchoalveolar lavage fluid in patients with biopsy proven sarcoidosis. *Sarcoidosis Vasc. Diffus. Lung Dis.* **32**, 305–312 (2016).

84. Hyldgaard, C., Kaae, S., Riddervold, M., Hoffmann, H. J. & Hilberg, O. Value of s-ACE, BAL lymphocytosis, and CD4+/CD8+ and CD103+CD4+/CD4+ T-cell ratios in diagnosis of sarcoidosis. *Eur. Respir. J.* **39**, 1037–1039 (2012).

85. Suchankova, M. *et al.* Triggering receptor expressed on myeloid cells-1 and 2 in bronchoalveolar lavage fluid in pulmonary sarcoidosis. *Respirology* **18**, 455–462 (2013).

86. Winterbauer, R. H. *et al.* Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. *Chest* **104**, 352–361 (1993).

87. D'Ippolito, R. *et al.* Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir. Med.* **98**, 977–983 (2004).

88. King, T. & , Mortenson, R. Cryptogenic organizing pneumonitis: the North American experience. *Chest* **102**, 8S-13S (1992).

89. Ward, K., O'connor, C., Odlum, C. & Fitzgerald, M. X. Prognostic value of bronchoalveolar lavage in sarcoidosis: the critical influence of disease presentation. *Thorax* **44**, 6–12 (1989).

90. Verstraeten, A. *et al.* Predictive value of bronchoalveolar lavage in pulmonary sarcoidosis. *Chest* **98**, 560–567 (1990).

91. Bjermer, L., Rosenhall, L., Angstrom, T. & Hallgren, R. Predictive value of bronchoalveolar lavage cell analysis in sarcoidosis. *Thorax* **43**, 284–288 (1988).

92. Laviolette, M., La Forge, J., Tennina, S. & Boulet, L. P. Prognostic value of bronchoalveolar lavage lymphocyte count in recently diagnosed pulmonary sarcoidosis. *Chest* **100**, 380–384 (1991).

93. Heron, M. *et al.* Evaluation of CD103 as a cellular marker for the diagnosis of pulmonary sarcoidosis. *Clin Immunol.* **126**, 338–44 (2008).

94. Danila, E., Norkuniene, J., Jurgauskiene, L. & Malickaite, R. Diagnostic role of BAL fluid CD4/CD8 ratio in different radiographic and clinical forms of pulmonary sarcoidosis. *Clin. Respir. J.* **3**, 214–221 (2009).

95. Von Bartheld, M. B. *et al.* Endosonography vs conventional bronchoscopy for the diagnosis of sarcoidosis: The GRANULOMA randomized clinical trial. *JAMA* **309**, 2457–2464 (2013).

96. Planck, A., Eklund, A. & Grunewald, J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **18**, 64–9 (2001).

97. Mota, P. C. *et al.* Diagnostic value of CD103 expression in bronchoalveolar lymphocytes in sarcoidosis. *Respir. Med.* **106**, 1014–1020 (2012).

98. Braun, R. K. *et al.* Phenotypic and molecular characterization of CD103+ CD4+ T cells in bronchoalveolar lavage from patients with interstitial lung diseases. *Cytometry B. Clin. Cytom.* **54**, 19–27 (2003).

99. van Eijk, M. *et al.* Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Int. Immunol.* **17**, 1505–1512 (2005).

100. Bargagli, E. *et al.* Human chitotriosidase: a sensitive biomarker of sarcoidosis. *J. Clin. Immunol.* **33**, 264–270 (2013).

101. Boot, R. G. *et al.* Plasma chitotriosidase and CCL18 as surrogate markers for granulomatous macrophages in sarcoidosis. *Clin. Chim. Acta.* **411**, 31–36 (2010).

102. Tercelj, M. *et al.* Chitotriosidase activity in sarcoidosis and some other pulmonary diseases. *Scand. J. Clin. Lab. Invest.* **69**, 575–578 (2009).

103. Harlander, M. *et al.* Serial chitotriosidase measurements in sarcoidosis--two to five year follow-up study. *Respir. Med.* **108**, 775–782 (2014).

104. Ostadkarampour, M. *et al.* Higher levels of interleukin IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Löfgren's syndrome. *Clin Exp Immunol.* **178**, 342–52 (2014).

105. Ten Berge, B. *et al.* Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. *Rheumatol.* **51**, 37–46 (2012).

106. Miedema, J. R. *et al.* Th17-lineage cells in pulmonary sarcoidosis and Löfgren's syndrome: Friend or foe? *J. Autoimmun.* **87**, 82–96 (2018).

107. Kaiser, Y. *et al.* Expanded lung T-bet+RORγT+ CD4+ T-cells in sarcoidosis patients with a favourable disease phenotype. *Eur. Respir. J.* **48**, 484–494 (2016).

108. Broos, C. E. *et al.* Impaired survival of regulatory T cells in pulmonary sarcoidosis. *Respir. Res.* **16**, (2015).

109. Meek, R. L., Eriksen, N. & Benditt, E. P. Murine serum amyloid A3 is a high density apolipoprotein and is secreted by macrophages. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 7949–7952 (1992).

110. Zhang, Y. *et al.* Preliminary characterizations of a serum biomarker for sarcoidosis by comparative proteomic approach with tandem-mass spectrometry in ethnic Han Chinese patients. *Respir. Res.* **14**, (2013).

111. Bargagli, E. *et al.* Analysis of serum amyloid A in sarcoidosis patients. *Respir. Med.* **105**, 775–780 (2011).

112. Baggiolini, M., Dewald, B. & Moser, B. Human chemokines: an update. *Annu. Rev. Immunol.* **15**, 675–705 (1997).

113. Agostini, C. *et al.* Role for CXCR6 and its ligand CXCL16 in the pathogenesis of T-cell alveolitis in sarcoidosis. *Am. J. Respir. Crit. Care Med.* **172**, 1290–1298 (2005).

114. Agostini, C. *et al.* Involvement of the IP-10 chemokine in sarcoid granulomatous reactions. *J Immunol.* **161**, 6413–20 (1998).

115. Katchar, K., Eklund, A. & Grunewald, J. Expression of Th1 markers by lung accumulated T cells in pulmonary sarcoidosis. *J. Intern. Med.* **254**, 564–571 (2003).

116. Miotto, D. *et al.* Expression of IFN-gamma-inducible protein; monocyte chemotactic proteins 1, 3, and 4; and eotaxin in TH1- and TH2-mediated lung diseases. *J. Allergy Clin. Immunol.* **107**, 664–670 (2001).

117. Nishioka, Y. *et al.* CXCL9 and 11 in patients with pulmonary sarcoidosis: a role of alveolar macrophages. *Clin. Exp. Immunol.* **149**, 317–326 (2007).

118. Piotrowski, W. J. *et al.* Chemokine receptor CXCR3 ligands in bronchoalveolar lavage fluid: associations with radiological pattern, clinical course, and prognosis in sarcoidosis. *Pol. Arch. Med. Wewn.* **124**, 395–402 (2014).

119. Su, R. *et al.* Interferon-inducible chemokines reflect severity and progression in sarcoidosis. *Respir. Res.* **14**, (2013).

120. Su, R. *et al.* Longitudinal analysis of sarcoidosis blood transcriptomic signatures and disease outcomes. *Eur. Respir. J.* **44**, 985–993 (2014).

121. Crouser, E. D. *et al.* Application of "omics" and systems biology to sarcoidosis research. *atsjournals.org* **14**, 445–451 (2017).

122. Crouser, E. D. *et al.* Gene expression profiling identifies MMP-12 and ADAMDEC1 as potential pathogenic mediators of pulmonary sarcoidosis. *Am. J. Respir. Crit. Care Med.* **179**, 929–938 (2009).

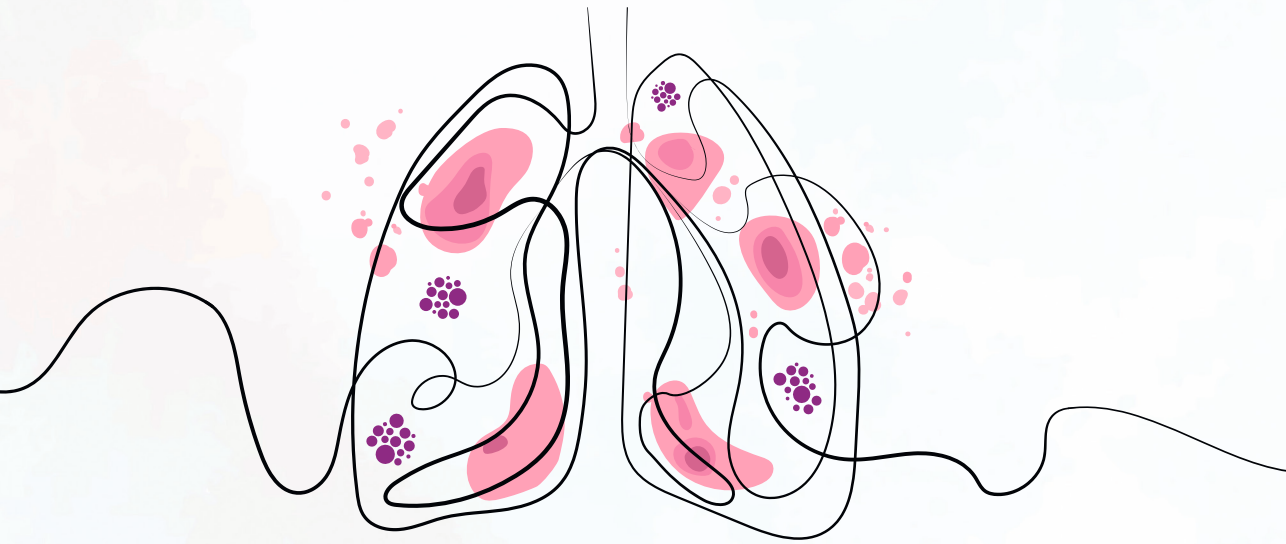
123. Koth, L. L. *et al.* Sarcoidosis blood transcriptome reflects lung inflammation and overlaps with tuberculosis. *Am. J. Respir. Crit. Care Med.* **184**, 1153–1163 (2011).

124. Lockstone, H. E. *et al.* Gene set analysis of lung samples provides insight into pathogenesis of progressive, fibrotic pulmonary sarcoidosis. *Am. J. Respir. Crit. Care Med.* **181**, 1367–1375 (2010).

125. Zhou, T. *et al.* Peripheral blood gene expression as a novel genomic biomarker in complicated sarcoidosis. *PLoS One* **7**, (2012).

126. Häggmark, A. *et al.* Proteomic profiling reveals autoimmune targets in sarcoidosis. *Am. J. Respir. Crit. Care Med.* **191**, 574–583 (2015).

127. Dragonieri, S. *et al.* An electronic nose discriminates exhaled breath of patients with untreated pulmonary sarcoidosis from controls. *Respir. Med.* **107**, 1073–1078 (2013).



CHAPTER 3

VALUE OF SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR AS A DIAGNOSTIC AND PREDICTIVE BIOMARKER IN SARCOIDOSIS

M.C. Schimmelpennink, M.J.R. Quanjel, A.D.M. Vorselaars, I.A. Wiertz, M. Veltkamp, C.H.M. Van Moorsel, J.C. Grutters

Expert. Rev. Respir. Med. 2020 Jul;14(7):749-756

ABSTRACT

Objectives

Differentiating between interstitial lung diseases (ILD) is challenging. Serum soluble interleukin 2 receptor (sIL-2R) is used as a diagnostic marker in sarcoidosis, but its diagnostic value has not yet been studied in other ILDs, like idiopathic pulmonary fibrosis (IPF) or hypersensitivity pneumonitis (HP). Also, the prognostic value of sIL-2R in sarcoidosis remains unknown.

Methods

This retrospective cohort study included 121 patients with sarcoidosis, 35 with chronic HP (cHP), 62 with IPF and 70 healthy controls. Serum sIL-2R levels were determined at diagnosis. Follow-up data were available for patients with chronic sarcoidosis (n=64) and patients with non-chronic sarcoidosis (n=29).

Results

Patients with sarcoidosis had higher sIL-2R levels (median 5418 pg/mL) than patients with cHP (median 4015 pg/mL, P=0.001) and IPF (median 4192 pg/mL, P<0.001). No differences were found in sIL-2R between patients with cHP and IPF. Logistic regression revealed that sIL-2R at diagnosis is a significant predictor of the development of chronic sarcoidosis (OR=2.1, P=0.030).

Conclusion

High levels of sIL-2R are suggestive of sarcoidosis, although a broad overlap exists in sIL-2R levels across sarcoidosis, cHP and IPF. High levels of sIL-2R might serve as a prognostic biomarker for chronicity.

INTRODUCTION

Interstitial lung diseases (ILDs) comprise a heterogeneous group of respiratory disorders characterised by diffuse parenchymal disease affecting the interstitium, and include sarcoidosis, hypersensitivity pneumonitis (HP) and idiopathic pulmonary fibrosis (IPF)[1].

Various ILDs can share certain clinical, radiological and pathological features, making differentiation between ILD challenging[2,3]. Achieving an accurate diagnosis often requires a multidisciplinary approach[4]. Biomarkers can be used as an extra tool to differentiate between different diagnoses. Sarcoidosis is a complex clinical diagnosis per exclusionem, meaning that the diagnosis is reached by a process of elimination of other diagnoses. Moreover, there are no pathognomonic markers for the diagnosis sarcoidosis. For decades researchers have been investigating biomarkers in order to ease the diagnostic process. Earlier studies established the value of soluble interleukin 2 receptor (sIL-2R) as biomarker in sarcoidosis[5–12]. Serum sIL-2R is a relatively new biomarker in sarcoidosis.

sIL-2R is a cytokine released by activated T-lymphocytes, and is therefore considered a marker of T-cell activation[13]. Sarcoidosis and HP are both granulomatous diseases initially presenting with lymphocytic alveolitis. IPF is a fibrotic disease characterised by a combination of subclinical epithelial injury and aberrant repair mechanism leading to deposition of fibrosis by (myo)fibroblasts.

Considering the pathogenesis of these different entities, we hypothesised that sIL-2R levels might be increased in patients with predominant inflammatory diseases, such as sarcoidosis or cHP, but less in patients with a predominant fibrotic disease like IPF. Consequently, sIL-2R could be a potential biomarker of interest to distinguish cHP from IPF.

Sarcoidosis is known for its heterogeneous spectrum of disease courses, varying from spontaneously resolving to chronic sarcoidosis, which can eventually lead to severe fibrosis[14]. It is clinically challenging to distinguish patients who will have spontaneously resolving disease from those who will have more chronic disease. Vorselaars et al.[15] showed that high levels of sIL-2R at initiation of infliximab therapy are predictive for relapse after discontinuation of infliximab. However, in this study the predictive value of sIL-2R at initiation of infliximab (third line treatment) was studied, whereas we study the predictive value of sIL-2R at the diagnosis. Furthermore, the value of sIL-2R as a biomarker for chronicity has not yet been well established.

In this study we provide insights in the use of sIL-2R as a diagnostic and prognostic marker in sarcoidosis. Previously, we demonstrated the use of sIL-2R as a diagnostic marker in sarcoidosis[5].

In the first part of this paper, we address the diagnostic value of sIL-2R to distinguish between patients with sarcoidosis, cHP and IPF. In the second part we discuss the potential of sIL-2R as a predictive prognostic biomarker in patients with sarcoidosis.

METHODS

Subjects

This retrospective study cohort included 121 patients with sarcoidosis (17 of whom had Löfgren's syndrome, LS), 35 patients with cHP and 62 patients with IPF. The control group consisted of 70 healthy subjects. In accordance with the ERS/ATS/WASOG statement[16], sarcoidosis was diagnosed when the clinical picture matched the histological evidence and after other granulomatous diseases had been excluded. No histological evidence was required for patients presenting with classical symptoms of LS (acute onset with bilateral hilar lymphadenopathy, fever, erythema nodosum (EN) and/or bilateral ankle arthritis)[17]. IPF was diagnosed according to the official ATS/ERS/JRS/ALAT statement of 2011[18]. At our tertiary referral center, the diagnoses of IPF and cHP were discussed for each patient by a multidisciplinary team including pulmonologists, radiologists and pathologists, all with expertise in ILD. Demographic data and smoking history were collected from medical records. For patients with sarcoidosis, additional information on Scadding stage and organ involvement was extracted from clinical files.Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius hospital[19]. The study was approved by the Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital (R05-08A) and all subjects gave written informed consent.

Soluble interleukin-2 receptor

At diagnosis, serum sIL-2R was quantitatively determined in serum using enzyme immunoassays according to the manufacturer's instructions (Diaclone; Sanquin, Amsterdam, the Netherlands). None of the patients were being treated for interstitial lung disease at the time of sIL-2R testing. Since renal insufficiency leads to a discrepant elevation of serum sIL-2R levels, patients with renal insufficiency (estimated glomerular filtration rate<60 ml/min/1.73m^2) were excluded[20]. The maximum period that elapsed between the diagnosis sarcoidosis, cHP and IPF and the time of sIL-2R testing was 4

months. A sIL-2R level of 3000 pg/mL is considered the upper limit of normal at our laboratory.

Follow-up

Patients with sarcoidosis were evaluated two years after diagnosis. Patients were included in the follow-up analysis when information was available on the clinical course of their disease up to two years after diagnosis. However, there were two exceptions also included: the patient was discharged from the outpatient clinical visits because all sarcoidosis activity had disappeared after respectively 16 and 20 months of follow-up. Follow-up data were retrospectively extracted from patient files, and included clinical symptomatology, treatment characteristics, lung function data, and, if applicable, serum markers, sIL-2R and angiotensin converting enzyme (ACE) and inflammatory activity on ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT). Forced vital capacity (FVC) at diagnosis and follow-up was available for 65 patients, and diffusing capacity for carbon monoxide (DLCOc) for 45 patients. In addition, the sIL-2R value after two years of follow-up was available for 74 patients, ACE for 75 patients and ^{18}F-FDG PET/CT for 25 patients. Based on clinical, functional and inflammatory markers two years after diagnosis, patients were divided into two groups: non-chronic sarcoidosis and chronic sarcoidosis. The definition of chronic sarcoidosis used in this study is outlined in table 1.

Table 1. Definition of chronic sarcoidosis

Chronic sarcoidosis was defined based on one or more of the following criteria:	**n=64, patients with chronic sarcoidosis**
Long-term use of systemic therapy (>2 years after diagnosis)	39 (60.9%)
Persistent inflammatory activity defined by increased biomarkers (ACE and sIL-2R) and/or increased inflammatory activity measured by SUVmax on PET	35 (54.6%)
Persistent symptomatic disease	12 (18.8%)
Chronic or progressive hypercalcemia	1 (1.6%)
Persistent or progressive severe pain due to small fibre neuropathy, ossal lesions, cutaneous lesions	5 (7.8%)
Progressive pulmonary sarcoidosis defined by a decrease in FVC>5% of predicted and/or a decrease in DLCO>5% of predicted 2 years after diagnosis	15 (23.1%)
Cardiac sarcoidosis: persistent arrhythmias, inflammatory activity on PET-scan and/or loss of left ventricle function	4 (6.3%)

ACE=angiotensin converting enzyme; sIL-2R=soluble interleukin 2 receptor; SUVmax=maximum standardised uptake value; PET=positron emission tomography; FVC=forced vital capacity; DLCO=diffusing capacity for carbon monoxide.

Statistics

Continuous parametric data are presented as means with standard deviation, whereas continuous non-parametric data are presented as medians with range. Categorical data are presented as numbers and percentages. Differences in median sIL-2R between groups were tested with the Mann-Whitney U test. All analyses were performed with IBM SPSS Statistics for Windows (version 24; Armonk, New York, USA). Receiver operating characteristic (ROC) curves were used to assess the diagnostic accuracy of sIL-2R to diagnose sarcoidosis, IPF and cHP. Logistic regression was used to determine the predictive value of sL-2R in the follow-up of patients with sarcoidosis.

RESULTS

Patients

The characteristics of our cohort are outlined in table 2. A total of 68 (58%) patients with sarcoidosis showed extrapulmonary involvement, in four of whom only extrapulmonary organs were involved. Extrapulmonary organs involved included heart, eyes, small nerve fibers, skin, central nervous system, bones, hypercalcemia, spleen, liver, extra thoracic lymph nodes, pleura, pancreas, bone marrow, kidney and salivary glands.

Table 2. Characteristics of patients with sarcoidosis, LS, IPF, cHP and healthy controls

		Sarcoidosis (n=104)	LS (n=17)	IPF (n=62)	cHP (n=35)	Healthy controls (n=70)
Age[a]		44.1 ± 10.8	34.8 ± 8.3	67.9 ± 9.0	59.9± 11.5	50.4 ± 13.3
Sex (male)		68 (65.4%)	8 (47.1%)	53 (85.5%)	16 (45.7%)	35 (50%)
Whites %		88 (84.6%)	17 (100%)	60 (96.8%)	33 (94.3%)	
Smoker	Yes	16 (15.4%)	3 (17.6%)	6 (9.7%)	0	
	No	54 (51.9%)	7 (41.2%)	11 (17.7%)	13 (37.1%)	
	Former	34 (32.7%)	7 (41.2%)	45 (72.6%)	22 (62.9%)	
Scadding stage	0	4 (3.8%)	-			
	I	45 (43.3%)	11 (64.7%)			
	II	37 (35.6%)	5 (29.4%)			
	III	5 (4.8%)	1 (5.9%)			
	IV	13 (12.5%)				
FVC (%pred)[a] at diagnosis		94.1 ± 20.7	102.1 ± 17.0	83.6 ± 21.0	75.5 ± 18.9	
FEV1 (%pred)[a] at diagnosis		86.8 ± 23.6	94.4 ± 20.5	84.8 ± 19.4	77.3± 18.3	
DLCOc (%pred)[a] at diagnosis		77.7 ± 17.2	85.1 ± 17.6	40.5 ± 11.5	42.0 ± 11.5	

[a]Mean ± standard deviation

LS=Löfgren's syndrome; IPF=idiopathic pulmonary fibrosis; cHP=chronic hypersensitivity pneumonitis; FVC=forced vital capacity; FEV1=forced expiratory volume in one second; DLCOc=diffusing capacity for carbon monoxide.

Soluble interleukin-2 receptor at diagnosis

The median sIL-2R values at diagnosis and the number of patients with increased sIL-2R values are presented for each patient group in table 3.

Table 3. Serum sIL-2R level at diagnosis in patients with sarcoidosis, LS, IPF and cHP

	Sarcoidosis (n=104)	LS (n=17)	IPF (n=62)	cHP (n=35)	Healthy controls (n=70)
Serum sIL-2R value at diagnosis; median (range)	5534 (1351-55000)	5682 (560 - 36000)	4192 (1452 - 9730)	4015 (1401 - 19700)	1028 (263 -2210)
Patients above normal range (n, percentage)	94 (90.4%)	16 (94.1%)	44 (71.0%)	28 (80.0%)	0 (0%)

Threshold for normal range is sIL-2R=3000 pg/ml.
LS=Lofgren's syndrome IPF=idiopathic pulmonary fibrosis; cHP=chronic hypersensitivity pneumonitis; sIL-2R=serum soluble interleukin 2 receptor.

All four patient groups had significantly higher median levels of sIL-2R than the healthy subjects (figure 1). The sensitivity of the sIL-2R level for diagnosing ILD was 83%, while sensitivities for sarcoidosis, LS, IPF and cHP were 90.4%, 94.4%, 71.0% and 80.0%, respectively.

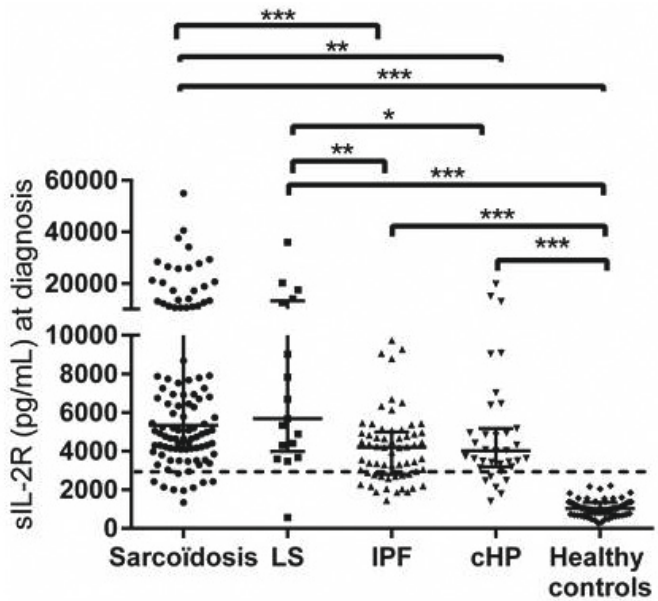


Figure 1. Serum sIL-2R value at diagnosis in patients with sarcoidosis, LS, IPF, cHP and healthy controls. **P < 0.01, ***P < 0.001. Threshold for normal range is sIL-2R = 3000 pg/ml. sIL-2R= soluble interleukin 2 receptor; LS = Löfgren's syndrome, IPF = idiopathic pulmonary fibrosis, cHP = chronic hypersensitivity pneumonitis.

The highest median sIL-2R was found in patients diagnosed with sarcoidosis (median 5534 pg/mL; range 1351–55 000). Patients with sarcoidosis showed significantly higher levels of sIL-2R than patients with IPF and patients with cHP, *P*<0.001 and *P*=0.001, respectively. No significant difference was found between patients with sarcoidosis and Löfgren's syndrome. Furthermore, no significant difference was found between patients with IPF and patients with cHP. As shown by figure 2 the median sIL-2R gradually increases with the Scadding stages. Patients with Scadding stage 0 had significant lower levels of sIL-2R than patients with Scadding stage I, II, IV, *P*<0.05. Furthermore, patients with Scadding stage IV showed significantly higher levels of sIL-2R than patients with Scadding stage I, *P*<0.05.

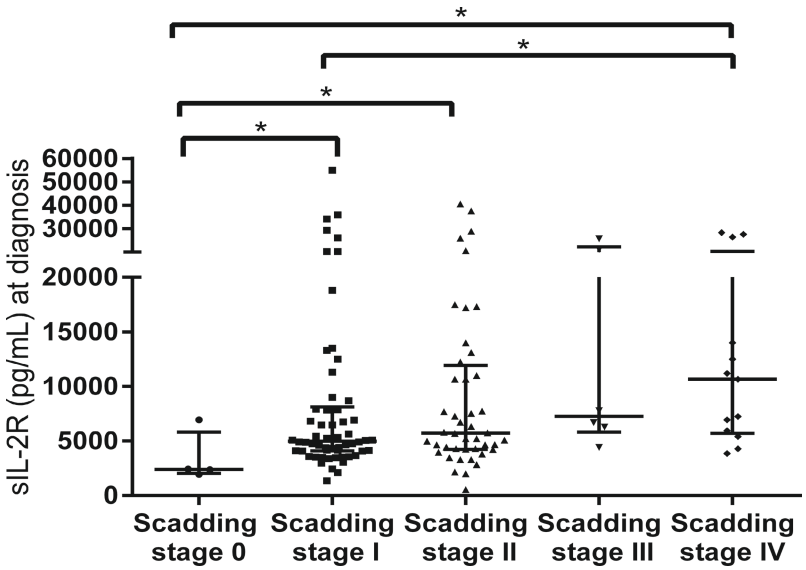


Figure 2. Median sIL-2R at diagnosis comparing different Scadding stages.
*P<0.05; sIL-2R = serum soluble interleukin 2 receptor.

ROC-curves were composed to determine the ability to discriminate between sarcoidosis (including Löfgren patients), IPF and cHP and the healthy control group. A sIL-2R level above 2300 pg/mL proved diagnostic for sarcoidosis in comparison with healthy controls, with a sensitivity of 0.95 and specificity of 1.00; area under the curve (AUC)=0.989 (95% confidence interval (CI) 1.0–1.0; *P*<0.001). Serum sIL-2R level above 1850 pg/mL proved diagnostic for IPF in comparison with healthy controls, with a sensitivity of 0.98 and a specificity of 0.94; AUC=0.993 (CI 1.0–1.0; *P*<0.001). A serum sIL-2R level above 2100 pg/mL proved diagnostic for cHP in comparison with healthy controls, with a sensitivity of 0.94 and a specificity of 0.91; AUC=0.993 (CI 1.0–1.0; *P*<0.001).

Values of sIL-2R above 5200 pg/mL discriminated between sarcoidosis and IPF or cHP, with a sensitivity of 0.53 and a specificity of 0.79; AUC= 0.713 (CI 0.6–0.8; *P*<0.001).

Patients with fibrosing sarcoidosis (i.e. patients with Scadding stage IV, n=13) had significantly higher sIL-2R levels at diagnosis than patients with cHP and those with IPF; *P*<0.001 and *P*<0.001 respectively (figure 3).

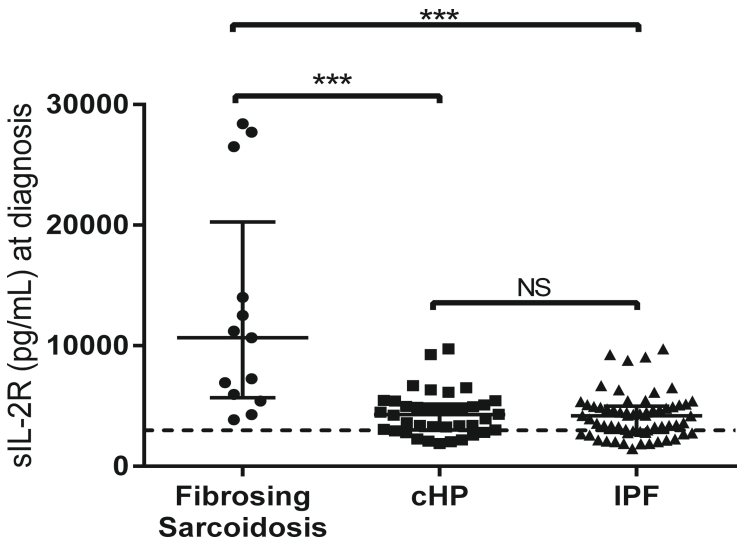


Figure 3. Serum sIL-2R value at diagnosis in patients with fibrosing sarcoidosis, IPF, and cHP
***P < 0.001. Threshold for normal range is sIL-2R = 3000 pg/ml. cHP = chronic hypersensitivity pneumonitis; IPF = idiopathic pulmonary fibrosis; NS= not significant.

Follow-up in sarcoidosis

Follow-up data at two years after diagnosis were available for 81 patients with sarcoidosis and 12 patients with LS. A total of 29 patients (31.2%) were evaluated as having non-chronic sarcoidosis, whereas 64 (68.8%) patients had chronic sarcoidosis. Median sIL-2R level at diagnosis was significantly higher in the chronic group than in the non-chronic group: 6831 (range, 1959–55 000) versus 4435 (range, 560–36000) (*P*=0.019) (figure 4).

Logistic regression yielded the odds ratio (OR) for the association between the sIL-2R level and the development of chronic sarcoidosis and the need for therapy. Values of sIL-2R were log-transformed to change the model from additive to multiplicative, in accordance with clinical interpretation, and to stabilise the variance. Logistic regression revealed that sIL-2R is a significant predictor of the development of chronic sarcoidosis, with an OR of 2.1 (CI 1.1–4.0; *P*=0.030).

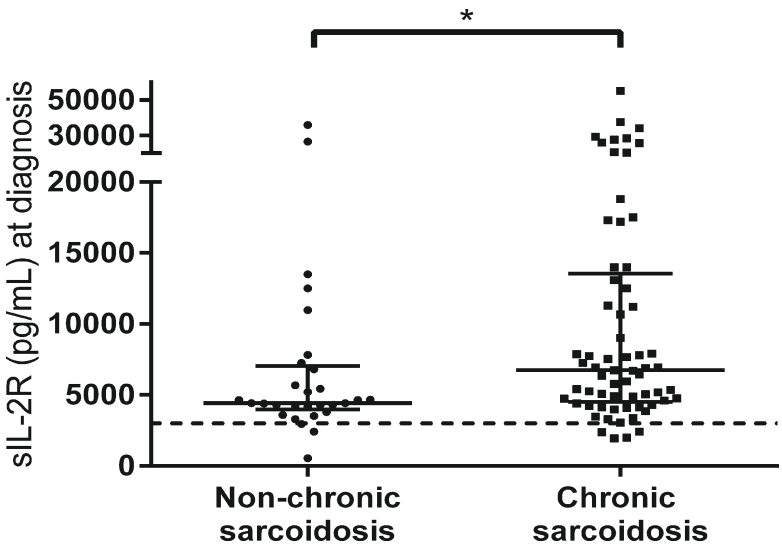


Figure 4. Serum sIL-2R value at diagnosis in patients with sarcoidosis, comparing the group with non-chronic disease and the group with chronic disease.
*P < 0.05. Threshold for normal range is sIL-2R = 3000 pg/ml.

ROC-curves showed that a sIL-2R level>4700 pg/mL was predictive of chronic sarcoidosis, with a sensitivity of 0.75 and a specificity of 0.62; AUC=0.653 (CI 0.5–0.8; *P*=0.020). More in detail, a sIL-2R level> 7000 pg/mL was predictive of the need for systemic treatment, with a sensitivity of 0.58 and a specificity of 0.78; AUC=0.672(CI 0.6 – 0.8; *P*=0.004). Follow-up data were available in 12 out of 13 patients with fibrotic sarcoidosis (i.e. Scadding stage IV). The non-chronic group included one fibrotic patient, whereas the chronic group included eleven fibrotic sarcoidosis patients. sIL-2R was not significantly different between patients with fibrotic sarcoidosis in the chronic group in comparison to non-fibrotic sarcoidosis in the chronic group, 11200 pg/mL versus 6709 pg/mL. A total of 19 patients underwent both a ^{18}F-FDG PET/CT scan at the diagnosis and two years after diagnosis. No significant correlation was found between the initial sIL-2R and the change in SUVmax. Furthermore, no correlation was found between the initial SUVmax and sIL-2R, nor did we find a significant correlation between the initial sIL-2R and the SUVmax at the ^{18}F-FDG PET/CT scan after two years.

DISCUSSION

In this study, we demonstrated that high sIL-2R level is highly sensitive for the diagnosis sarcoidosis. In addition, high sIL-2R levels at diagnosis were found to be predictive for developing chronic sarcoidosis. Interestingly, we found a significantly increased

level of sIL-2R in patients with IPF, a predominant fibrotic disease, and no difference when compared to patients with cHP, a predominant inflammatory-driven disease. Our results show that sIL-2R is not specific for sarcoidosis. Therefore, sIL-2R is suitable as prognostic biomarker, but not as diagnostic biomarker in sarcoidosis when other ILDs are in the differential diagnosis. On the other hand, lung parenchyma is not always involved in sarcoidosis[21]. Often, patients present with hilar adenopathy without involvement of parenchyma (Scadding stage I), in those cases lymphoma or tuberculosis is a more likely alternative diagnosis[22,23]. Unfortunately, increased levels of sIL-2R are also found in patients with tuberculosis[24,25] and lymphoma[26]. Therefore, sIL-2R is not a suitable biomarker to differentiate between sarcoidosis and tuberculosis or lymphoma. However, although sIL-2R is not specific for the diagnosis sarcoidosis, our study shows that patients with sarcoidosis have significantly higher levels of sIL-2R in comparison to the other ILDs.

In our cohort, 90% of the sarcoidosis patients showed increased levels of sIL-2R at diagnosis. Which is even higher than 79% sensitivity what was found earlier in the study of Grutters in our research center[5]. We found an AUC of 0.989 for the diagnosis sarcoidosis in comparison with healthy subjects. This result is similar to the AUC of 1.00 found in the recently published study by Uysal[11]. The sensitivity of sIL-2R for diagnosing sarcoidosis is higher than the sensitivity of the conventional biomarker of sarcoidosis ACE, 40-80%[27–29]. Moreover, the sensitivity of ACE is limited by a polymorphism in the ACE gene[30] and the use of ACE-inhibitors[31]. Although sarcoidosis patients showed significantly higher levels of sIL-2R than patients with IPF and cHP, a broad overlap in sIL-2R levels was found between different ILDs. Therefore, sIL-2R is not suitable as an extra differentiating tool during the diagnostic process. The study by Reynolds et al.[32] and the study of Tsutsumi et al.[33] also showed increased levels of sIL-2R in the BAL fluids and sera of patients with IPF and cHP. However, these studies are outdated because they were performed before the international guidelines of IPF were published in 2011[18]. The characteristics of the included IPF patients in our cohort differs from IPF cohorts before the guidelines, because of the switch from consensus-based to evidence-based guidelines.

In the second part of our study, we investigated the prognostic value of sIL-2R in sarcoidosis. Our results showed that high sIL-2R levels (>4700pg/ml) were predictive for developing chronic sarcoidosis. More in detail, high sIL-2R levels were also predictive for the need of systemic therapy. Ziegenhagen and colleagues[34] performed a retrospective study investigating predictive markers in sarcoidosis. In this study was also found that sIL-2R was predictive for progressing disease, however the mean follow-up time was only 6 months. No significant difference exists between median sIL-2R in fibrotic sarcoidosis patients versus non-fibrotic sarcoidosis patients in the chronic group. Although we see

a trend towards higher levels in patients with fibrotic sarcoidosis, this group is probably too small (n=11) to detect significant differences.

We demonstrated that patients with higher sIL-2R levels were more likely to evolve chronic sarcoidosis. Paradoxically, we were not able to demonstrate significant differences in basal values of sIL-2R between sarcoidosis and LS. Another remarkable finding is that six patients with LS evolved chronic disease. No significant differences were found between LS patients with and without chronic disease. LS is a clinical phenotype of sarcoidosis that is associated with a favourable prognosis. The typical clinical presentation of LS with an acute onset, fever, erythema nodosum and arthritis suggests higher inflammatory activity than in patients with a more insidious onset of disease. On the basis of this hypothesis one should expect even higher levels of sIL-2R in patients with LS in comparison to non-LS patients. In the study of Ziegenhagen et al.[35] no difference in sIL-2R between patients with LS in comparison to non-LS stable sarcoidosis patients was found, but patients with progressive non-LS sarcoidosis showed markedly higher levels of sIL-2R than patients with LS. Planck and colleagues[36] found no difference in sIL-2R when comparing HLA DR 17 positive patients -which is associated with LS- to HLA DR17 negative patients; these results are in line with the findings in our cohort. Prasse et al.[37] compared serum sIL-2R levels in different clinical phenotypes of sarcoidosis and found the highest level in patients with acute sarcoidosis and those needing systemic therapy. We partly confirm these results by demonstrating higher sIL-2R levels in patients with the need for systemic therapy. Vorselaars and colleagues[15] showed that high sIL-2R at start of third line treatment with infliximab was predictive for relapse after discontinuation of therapy. Another study of Vorselaars demonstrated that sIL-2R levels reflected lung function change during methotrexate therapy[38]. For future research it might be interesting to investigate the prognostic value of sIL-2R in combination with other promising prognostic biomarkers, such as chitotriosidase (CTO). CTO has been proposed as prognostic biomarker, for the reason that CTO is associated with fibrotic sarcoidosis and persistent disease[39]. This enzyme, produced by macrophages[40], is an indicator of the severity of sarcoidosis[41–43] and correlates well with the clinical course of sarcoidosis[44,45]. In a recent study it was demonstrated that the combined application of ACE and CTO improves the diagnostic sensitivity as well as specificity in sarcoidosis[46]. Prognostic capacity was not studied. Therefore in future studies it has to be demonstrated if the combined use of for example sIL-2R and CTO might enhance the prognostic value. A strong aspect of this study is that it was performed in a tertiary hospital and all diagnoses were carefully discussed in a disciplinary team with experts in the field of ILD. On the other hand, our study population is biased due to the fact that all patients are included in our tertiary referral center. Another limitation of this study is its retrospective design causing unstructured

follow-up data in sarcoidosis patients. However, we feel that our results are a realistic reflection of our sarcoidosis population.

From this study, it appears that sIL-2R and thus T-lymphocytes are involved in not only sarcoidosis and cHP, but also in IPF. Both sarcoidosis and cHP are characterised by lymphocytosis. We hypothesised that sIL-2R would be a marker for inflammatory diseases such as sarcoidosis and cHP, while IPF is more a fibrotic disease. Surprisingly, patients with fibrotic sarcoidosis showed higher levels than patients with non-fibrotic sarcoidosis arguing against our hypothesis. However, in sarcoidosis (as well as in cHP), fibrosis is a consequence of severe inflammation explaining lymphocytosis accompanied by excessive sIL-2R production[47]. Whereas it is likely to assume that in IPF lymphocytosis is an independent manifestation or a consequence rather than a cause of disease[48].

Our study results promote the use of sIL-2R as biomarker in the monitoring and clinical guidance of sarcoidosis. More in detail, our results suggest that patients with high levels of sIL-2R at diagnosis should be monitored closely as they appear to have more persistent and progressive disease. Unfortunately, despite the high sensitivity, in all three ILDs elevated sIL-2R levels were found. Advanced pulmonary sarcoidosis can mimic the radiologic pattern of IPF and cHP, however we demonstrated that fibrotic sarcoidosis patients had significantly higher levels of sIL-2R than patients with IPF and cHP. Moreover, often sarcoidosis does not present with the same pattern of parenchyma involvement as IPF and cHP.

CONCLUSION

In conclusion, our results provide new information about the diagnostic and prognostic value of sIL-2R in different interstitial lung diseases. High sIL-2R levels are highly sensitive for sarcoidosis, although not specific. Furthermore, high sIL-2R at time of diagnosis is prognostic for developing chronic sarcoidosis. Finally, even in a predominant fibrotic disease like IPF elevated levels of sIL-2R can be found making it not a suitable marker in distinguishing IPF from cHP.

REFERENCES

1. Bourke, S. J. Interstitial lung disease: progress and problems. *Postgrad. Med. J.* **82**, 494–499 (2006).
2. Morell, F. *et al.* Chronic hypersensitivity pneumonitis in patients diagnosed with idiopathic pulmonary fibrosis: a prospective case-cohort study. *Lancet.Respiratory Med.* **1**, 685–694 (2013).
3. Silva, C. I. *et al.* Chronic hypersensitivity pneumonitis: differentiation from idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia by using thin-section CT. *Radiology* **246**, 288–297 (2008).
4. Richeldi, L. *et al.* The characterisation of interstitial lung disease multidisciplinary team meetings: a global study. *ERJ open Res.* **5**, 10.1183/23120541.00209-2018. eCollection 2019 Apr (2019).
5. Grutters, J. C. *et al.* Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest* **124**, 186–195 (2003).
6. Gungor, S. *et al.* Conventional markers in determination of activity of sarcoidosis. *Int. Immunopharmacol.* **25**, 174–179 (2015).
7. Keijsers, R. G. *et al.* 18F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis. *Eur. J. Nucl. Med. Mol. Imaging* **36**, 1131–1137 (2009).
8. Lawrence, E. C. *et al.* Elevated serum levels of soluble interleukin-2 receptors in active pulmonary sarcoidosis: relative specificity and association with hypercalcemia. *Sarcoidosis* **4**, 87–93 (1987).
9. 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* **98**, 1125–1129 (1990).
10. Miyoshi, S. *et al.* Comparative evaluation of serum markers in pulmonary sarcoidosis. *Chest* **137**, 1391–1397 (2010).
11. Uysal, P. *et al.* YKL-40, Soluble IL-2 Receptor, Angiotensin Converting Enzyme and C-Reactive Protein: Comparison of Markers of Sarcoidosis Activity. *Biomolecules* **8**, 10.3390/biom8030084 (2018).
12. Ziegenhagen, M. W., Rothe, M. E., Schlaak, M. & Muller-Quernheim, J. Bronchoalveolar and serological parameters reflecting the severity of sarcoidosis. *Eur. Respir. J.* **21**, 407–413 (2003).
13. Rubin, L. A. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol. (Baltimore, Md. 1950)* **135**, 3172–3177 (1985).
14. Pereira, C. A., Dornfeld, M. C., Baughman, R. & Judson, M. A. Clinical phenotypes in sarcoidosis. *Curr. Opin. Pulm. Med.* **20**, 496–502 (2014).
15. Vorselaars, A. D. *et al.* Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur. Respir. J.* **43**, 602–609 (2014).
16. Hunninghake, G. W. *et al.* ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **16**, 149–173 (1999).

17. Karakaya, B., Kaiser, Y., van Moorsel, C. H. M. & Grunewald, J. Lofgren's Syndrome: Diagnosis, Management, and Disease Pathogenesis. *Semin. Respir. Crit. Care Med.* **38**, 463–476 (2017).

18. Raghu, G. *et al.* An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am. J. Respir. Crit. Care Med.* **183**, 788–824 (2011).

19. Harris, P. A. *et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J. Biomed. Inform.* **42**, 377–381 (2009).

20. Verwoerd, A. *et al.* Discrepant elevation of sIL-2R levels in sarcoidosis patients with renal insufficiency. *Eur. Respir. J.* **46**, 277–280 (2015).

21. Schupp, J. C. *et al.* Phenotypes of organ involvement in sarcoidosis. *Eur. Respir. J.* **51**, 10.1183/13993003.00991-2017. Print 2018 Jan (2018).

22. Lee, C. U., Chong, S., Choi, H. W. & Choi, J. C. Quantitative image analysis using chest computed tomography in the evaluation of lymph node involvement in pulmonary sarcoidosis and tuberculosis. *PLoS One* **13**, e0207959 (2018).

23. Yu, C. *et al.* Is SUVmax Helpful in the Differential Diagnosis of Enlarged Mediastinal Lymph Nodes? A Pilot Study. *Contrast Media Mol. Imaging* **2018**, 3417190 (2018).

24. Ito, M. *et al.* Soluble interleukin-2 receptors in pulmonary tuberculosis. *Jpn. J. Med.* **29**, 583–586 (1990).

25. Brown, A. E., Rieder, K. T. & Webster, H. K. Prolonged elevations of soluble interleukin-2 receptors in tuberculosis. *Am. Rev. Respir. Dis.* **139**, 1036–1038 (1989).

26. Khaled, H. M., Abdelhamid, T. M., Abu-Taleb, F. M., El-Hifnawi, N. M. & Waley, A. B. Impact of serum levels of IL-18 and soluble IL-2 receptor on the clinical outcome of patients with diffuse large B-cell lymphoma treated with R-CHOP regimen. *Futur. Sci. OA* **5**, FSO414 (2019).

27. De Smet, D. *et al.* Use of likelihood ratios improves interpretation of laboratory testing for pulmonary sarcoidosis. *Am. J. Clin. Pathol.* **134**, 939–947 (2010).

28. Popevic, S. *et al.* Verifying Sarcoidosis Activity: Chitotriosidase versus ACE in Sarcoidosis - a Case-control Study. *J. Med. Biochem.* **35**, 390–400 (2016).

29. Ungprasert, P., Carmona, E. M., Crowson, C. S. & Matteson, E. L. Diagnostic Utility of Angiotensin-Converting Enzyme in Sarcoidosis: A Population-Based Study. *Lung* **194**, 91–95 (2016).

30. Rigat, B. *et al.* An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J. Clin. Invest.* **86**, 1343–1346 (1990).

31. Krasowski, M. D. *et al.* Ordering of the Serum Angiotensin-Converting Enzyme Test in Patients Receiving Angiotensin-Converting Enzyme Inhibitor Therapy: An Avoidable but Common Error. *Chest* **148**, 1447–1453 (2015).

32. Reynolds, S. P., Jones, K. P., Edwards, J. H. & Davies, B. H. Immunoregulatory proteins in bronchoalveolar lavage fluid. A comparative analysis of pigeon breeders' disease, sarcoidosis and idiopathic pulmonary fibrosis. *Sarcoidosis* **6**, 125–134 (1989).

33. Tsutsumi, T. *et al.* Soluble interleukin-2 receptor in blood from patients with sarcoidosis and idiopathic pulmonary fibrosis. *Sarcoidosis* **11**, 102–109 (1994).

34. Homolka, J. *et al.* Systemic immune cell activation in a subgroup of patients with idiopathic pulmonary fibrosis. *Respiration.* **70**, 262–269 (2003).

35. Ziegenhagen, M. W. *et al.* Sarcoidosis: TNF-alpha release from alveolar macrophages and serum level of sIL-2R are prognostic markers. *Am. J. Respir. Crit. Care Med.* **156**, 1586–1592 (1997).

36. Planck, A., Eklund, A. & Grunewald, J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **18**, 64–9 (2001).

37. Prasse, A. *et al.* Phenotyping sarcoidosis from a pulmonary perspective. *Am. J. Respir. Crit. Care Med.* **177**, 330–336 (2008).

38. Vorselaars, A. D. *et al.* ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. *Respir. Med.* **109**, 279–285 (2015).

39. Bennett, D. *et al.* Chitotriosidase: A biomarker of activity and severity in patients with sarcoidosis. *Respir. Res.* **21**, 6 (2020).

40. van Eijk, M. *et al.* Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Int. Immunol.* **17**, 1505–1512 (2005).

41. Bargagli, E. *et al.* Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. *Scand. J. Clin. Lab. Invest.* **68**, 479–483 (2008).

42. Bargagli, E. *et al.* Human chitotriosidase: a sensitive biomarker of sarcoidosis. *J. Clin. Immunol.* **33**, 264–270 (2013).

43. Tercelj, M. *et al.* Chitotriosidase activity in sarcoidosis and some other pulmonary diseases. *Scand. J. Clin. Lab. Invest.* **69**, 575–578 (2009).

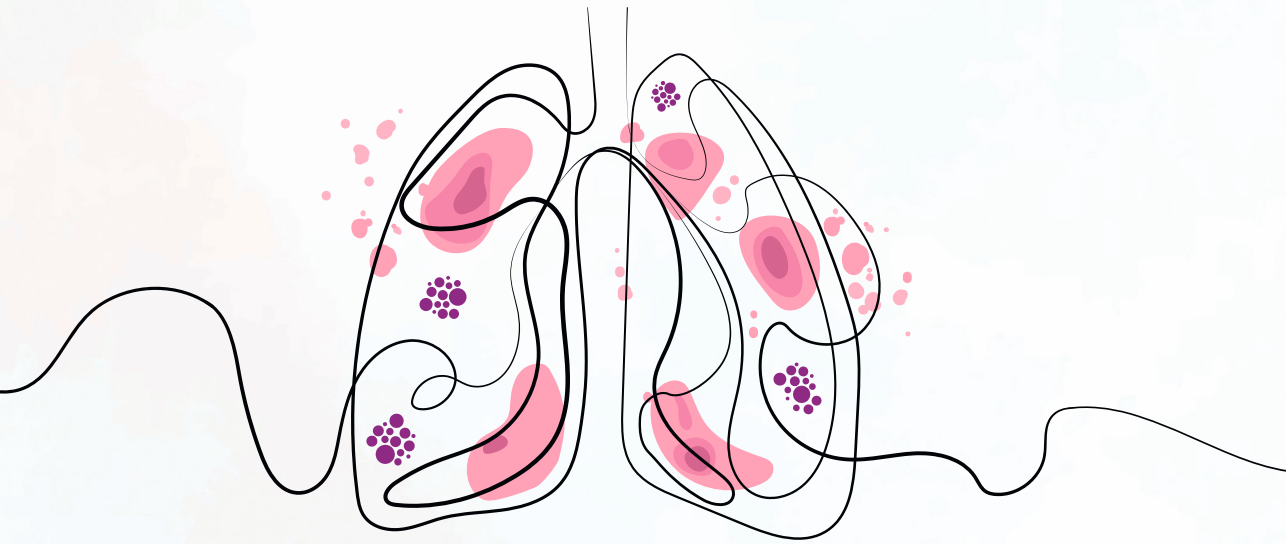
44. Boot, R. G. *et al.* Plasma chitotriosidase and CCL18 as surrogate markers for granulomatous macrophages in sarcoidosis. *Clin. Chim. Acta.* **411**, 31–36 (2010).

45. Harlander, M. *et al.* Serial chitotriosidase measurements in sarcoidosis--two to five year follow-up study. *Respir. Med.* **108**, 775–782 (2014).

46. Enyedi, A. *et al.* Combined application of angiotensin converting enzyme and chitotriosidase analysis improves the laboratory diagnosis of sarcoidosis. *Clin. Chim. Acta.* **500**, 155–162 (2020).

47. Luzina, I. G., Todd, N. W., Iacono, A. T. & Atamas, S. P. Roles of T lymphocytes in pulmonary fibrosis. *J. Leukoc. Biol.* **83**, 237–244 (2008).

48. Heukels, P., Moor, C. C., von der Thusen, J. H., Wijsenbeek, M. S. & Kool, M. Inflammation and immunity in IPF pathogenesis and treatment. *Respir. Med.* **147**, 79–91 (2019).



CHAPTER 4

BRONCHOALVEOLAR LAVAGE CHARACTERISTICS CORRELATE WITH HLA TAG SNPS IN PATIENTS WITH LÖFGREN'S SYNDROME AND OTHER SARCOIDOSIS

B. Karakaya, M.C. Schimmelpennink, L. Kocourkova, J.J. van der Vis, B. Meek, J.C. Grutters, M. Petrek, C.H.M. van Moorsel

Clin Exp Immunol. 2019 May;196(2):249-258.

ABSTRACT

Objective

Genetic susceptibility for sarcoidosis and Löfgren's syndrome (LS) has been associated with prognosis. Human leukocyte antigen (HLA)-*DRB1**03 is over-represented in LS, and is associated with a good prognosis, whereas *HLA-DRB1**15 positive patients have a more chronical course of sarcoidosis. These *HLA-DRB1* types can be easily tagged by single nucleotide polymorphisms (SNPs). Our aim was to evaluate the association between these tag SNPs and bronchoalveolar lavage (BAL) characteristics.

Methods

In 29 patients both complete *HLA-DRB1** locus genotyping and SNP tagging was performed in parallel. *HLA-DRB1* type was inferred from the presence of *03 tag rs2040410 allele A and referred to as *03. *HLA-DRB1**15 was inferred from the presence of tag SNP rs3135388 allele A and referred to as*15. For BAL analysis, 122 patients with LS and 165 patients with non-LS sarcoidosis were included. BAL lymphocyte subsets were analysed by flow cytometry.

Results

The presence of tag SNPs completely corresponded with *HLA-DRB1**03/*15 genotypes in all 29 patients in whom both *HLA-DRB1** genotyping and SNP tagging was performed. In all patients together, *03+/*15- patients showed higher CD4+/CD8+ ratio than *03-/*15+ (p=0.004) and *03-/*15- (p=0.001). LS patients with *03+/*15- had a lower BAL lymphocyte count compared to *03-/*15+ patients (p=0.011). Non-LS sarcoidosis patients with *03+/*15- patients showed a decreased CD103+CD4+/CD4+ ratio compared to *03-/*15+ patients (p=0.045) and *03-/*15- patients (p=0.018).

Conclusion

We found that *HLA-DRB1**03 and *HLA-DRB1**15 can be approximated by genotyping of tag SNPs and corresponds with the degree of lymphocytosis and cell phenotypes in BAL in both LS and non-LS sarcoidosis patients.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder with a wide variation in clinical manifestation and disease outcome and is mediated primarily by CD4+ T-helper (Th) cells. The course and prognosis may correlate with the mode of onset and extent of the disease. Löfgren's syndrome (LS), first described by Sven Löfgren, is an acute form of sarcoidosis that is associated with a favourable prognosis[1–5].

Genetic variation in the Human Leukocyte Antigen (in humans, HLA) region has been associated with the clinical course of sarcoidosis patients. *HLA-DRB1**03 allele is associated with a spontaneously resolving course of the disease and is associated with LS[6–9]. In more than 90% of the *HLA-DRB1**03 positive Löfgren patients the disease resolves within two years[6]. By contrast, *HLA-DRB1**03 negative patients commonly have non-resolving disease. In line with this, non-Löfgren's syndrome (non-LS) sarcoidosis patients carrying *HLA-DRB1**15 have an increased risk for a chronic course of the disease[10–12]. Frequencies of different HLA genotypes vary between ethnicities. For example, *HLA-DRB1**03 is very uncommon in Japan, whereas in a Dutch cohort *DRB1**03 was found in 40% of the sarcoidosis patients[9]. Furthermore, *HLA-DRB1**15 was found to be a risk factor for sarcoidosis in a white population, but not in a black sarcoidosis population[13]. *HLA-DRB1* typing can be used for risk stratification in sarcoidosis patients. However, full *HLA-DRB1* typing requires multiple steps using either sequence-specific oligonucleotides, polymerase chain reaction (PCR) primers or even sequencing, which makes this method laborious and expensive. In the last decade, studies have shown that particular *HLA-DRB1* types can be tagged by single nucleotide polymorphisms (SNPs) in linkage disequilibrium with *DRB1* genotypes[14]. In contrast to full *HLA-DRB1* genotyping, SNP tagging is a simple procedure. Bakker et al. found that SNP rs2040410 allele A and SNP rs3135388 have been associated with *HLA-DRB1**0301 and *HLA-DRB1**1501, respectively. Furthermore, in patients with diabetes type I SNP rs2040410 was found to identify *HLA-DRB1**0301 with great sensitivity and specificity, while SNP rs3135388 has been associated with systemic lupus erythematosus and multiple sclerosis[15]. Tagging is less time-consuming and less expensive compared with complex full HLA-analysis. In other systemic diseases, tag SNPs have also been investigated in order to capture HLA genotypes. For example, in a Japanese cohort with patients with type I diabetes tag SNP rs3129888 captured haplotype *HLA-DRB1**0802 with high sensitivity and specificity. To our knowledge, we performed the first use of these tag SNPs in sarcoidosis. For this reason, we validated the *HLA-DRB1* tag SNPs in patients with LS.

Analysis of bronchoalveolar lavage (BAL) can be used to support the diagnosis of sarcoidosis by demonstrating increased total cell count, lymphocytosis and an

increased CD4+/CD8+ ratio[16,17]. More recent studies have proved that a decreased CD103+CD4+/CD4+ ratio in the BAL is an additional reliable tool in the diagnostic work-up of sarcoidosis patients[18,19]. Previous reports have described characteristics of BAL in different clinical phenotypes of sarcoidosis, particularly LS versus non-LS sarcoidosis patients[20]. However, variability between patients is considerable. A few studies have investigated the role of *HLA-DRB1**03 on BAL outcomes[21,22]. However, in most of these studies no subanalysis was performed on patients with LS. Furthermore, to our knowledge, the influence of *HLA-DRB1**1501 on the BAL outcomes has not been described. This is of particular interest because *HLA-DRB1**1501 associates with a worse prognosis and clinically it is most important to identify these patients. We investigated the CD103+CD4+/CD4+ ratio in BAL and compared this in different HLA genotypes. This ratio has not yet been described in different HLA genotypes.

Next to validation of the tag SNPs for *HLA-DRB1**03 and -*DRB1**15, we investigated if these tags correlate with BAL cell phenotypes in patients with LS and non-LS.

METHODS

All patients were diagnosed in accordance with the American Thoracic Society/ European Respiratory Society/ World Association of Sarcoidosis and other Granulomatous Disorders (ATS/ERS/WASOG) consensus statement on sarcoidosis[23]. Patients with LS presented with the classic symptoms of acute onset with bilateral hilar lymphadenopathy, fever, erythema nodosum (EN) and/or bilateral ankle arthritis[5]. A total of 126 LS patients were included in our cohort: 122 patients with BAL and HLA typing/tag alleles and four patients with only HLA typing/tag alleles. In the first part of our study, the association of *HLA-DRB1**03 and *15 with the tag alleles was examined in 29 unrelated Dutch patients with LS. The tagging was confirmed with high-resolution HLA typing, i.e. *HLA-DRB1**0301 and *HLA-DRB1**1501. For the second part of the study, BAL and presence of the *03 and *15 tag alleles were analysed in 122 patients with LS (23 patients from the above-mentioned LS cohort), 165 patients with non-LS and 53 healthy controls. All included patients visited St. Antonius ILD Centre of Excellence, a tertiary referral center for interstitial lung diseases.

Therefore, we accepted a maximum duration of four months between diagnosis and performing BAL in LS patients and non-LS patients. Data from patients were collected retrospectively from medical charts and the following parameters were recorded: gender, age at diagnosis, Scadding (chest X-ray) stage, corticosteroid use and smoking status. At the time of BAL collection 10 LS patients used corticosteroids, 9 oral and 1 by

inhalation. Regarding non-LS patients, 17 patients used corticosteroids, 4 oral and 13 by inhalation at the time of BAL. The study was approved by the Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital (R05-08A) and all subjects gave written informed consent.

Genotyping for HLA-DRB1, SNP tags and bronchoalveolar lavage

Genomic DNA was extracted from peripheral blood of each individual using standard methods. In 29 patients, *HLA-DRB1* locus was genotyped using PCR-reverse sequence-specific oligonucleotides (SSO#) methodology (LABType® SSO, One Lambda Inc. Canoga Park CA USA). In all subjects, tag SNPs rs2040410A and rs3135388A were used to capture *HLA-DRB1**0301 and *HLA-DRB1**1501, respectively[15]. For rs2040410 genotyping, a restriction fragment length polymorphism (RFLP) assay was performed. Briefly, we amplified a 228 basepairs (bp) PCR product (forward primer: 5'- GTCTTTGGCTGGAGGCATTG-3'; reverse primer: 5'-GACTCATGGCTTGCCCCATA-3') and the product was digested by the restriction enzyme BsrGI (New England Biolabs, Ipswich, MA, USA) for 16h at 37°C. The products were separated on a 2% agarose gel. The band sizes were as follows for each genotype: AA=228, AG=49, 179 and 228, and GG=49 and 179 bp. To identify genotype rs3135388 tag, a custom designed taqman SNP genotyping assay was performed on an ABI 7500Fast analyser (Applied Biosystems, Foster City, CA) according to standard methodology. From this point forward in our report, the tagging alleles for *HLA-DRB1**03 and *HLA-DRB1**15 are abbreviated as *03 and *15, respectively.

All patients and healthy subjects underwent bronchoscopy and BAL procedure with a flexible bronchoscope according to the guidelines of the ERS[24,25], as described previously[18]. To determine lymphocyte subsets in peripheral blood and BAL cellular fraction, flow cytometry was performed as described previously[18].

Statistics

SPSS version 24 and Graphpad prism software version 6.05 were used for analysis. Data are expressed as median; upper (maximum) and lower (minimum) values. The non-parametric Mann-Whitney U-test and Kruskal-Wallis test were computed to test for differences in medians. The Chi-square test was used to compare proportions.

RESULTS

In a total of 29 patients, both full *HLA-DRB1* genotypes and tag SNPs rs2040410 and rs3135388 were determined. Characteristics of the patients are shown in table 1.

Table 1. Characteristics of patients with LS, non-LS, and healthy controls

		LS (n=126)	Non-LS (n=165)	P-value	Healthy subjects (n=53)
Age		34 (±15)	39 (±17)	0.003	22 (±21)
Gender male		48 (38%)	94 (57%)	0.001	29 (55%)
Caucasian race		124 (98%)	152 (93%)	0.025	
Tag DRB1*03 positive		89 (70%)	26 (16%)	<0.001	17 (33%)
Tag DRB1*15 positive		31 (24%)	48 (29%)	NS	10 (19%)
Smoking	Never	80/125 (63%)	87/164 (53%)		26/49 (53%)
	Current	20/125 (16%)	34/164 (21%)	NS	19/49 (39%)
	Ex	25/125 (20%)	43/164 (26%)		4/49 (8%)
Scadding stage	0	3/104 (3%)	6/145 (4%)		
	I	93/104 (89%)	68/145 (47%)		
	II	8/104 (8%)	43/145 (30%)	<0.001	
	III	-	24/145 (17%)		
	IV	-	4/145 (4%)		

Age is median± interquartile range (IQR) LS=Löfgren's syndrome, non-LS=non-Löfgren's syndrome, Bronchoalveolar lavage (BAL) was performed in 122 of 126 patients with LS. Furthermore, smoking history was available from 125 patients with LS, 164 non-LS, and 49 healthy subject. Scadding stage was available from 104 patients with LS and 110 patients with non-LS.
P-value is based on the comparison between LS and non-LS.

Table 2 shows the validation of the association between the *HLA-DRB1* genotypes and the tags *03 and *15 in Dutch patients. The tag alleles and the corresponding *HLA-DRB1* alleles overlapped completely. *HLA-DRB1**03 positivity corresponded with presence of the A allele of the tag SNP rs2040410, while *HLA-DRB1**15 positivity corresponded with the A allele of the tag SNP rs3135388.

Table 2. Validation of association between *HLA-DRB1* genotyping and tag SNPs rs2040410 and rs3135388

DRB1*03	**rs2040410**	**Allele A**	***DRB1*15***	**rs3135388**	**Allele A**	***DRB1*03***	***DRB1*15***	
	positive	negative		positive	negative		positive	negative
positive	16	0	positive	13	0	positive	4	12
negative	0	13	negative	0	16	negative	9	4

Bronchoalveolar lavage outcomes in all patients

BAL was performed in a total of 122 patients with LS, 165 patients with non-LS and 53 healthy controls. All BAL samples were obtained and analysed in our hospital. We compared BAL results of all patients (both LS and non-LS), subdividing patients on the basis of HLA tags *03 and *15 (table 3 and figures 1 and 2).

Table 3. Bronchoalveolar lavage and peripheral blood (PB) findings of all patients categorized in four *HLA-DRB1* genotypes: *03+/*15-, *03-/*15+, *03-/*15-, *03+/*15+

Sarcoidosis patients	***03+/*15-**		***03-/*15+**		***03- /*15-**		***03+ / *15+**		**P**
(LS and non-LS)	n	Median (upper-lower value)	n	Median (upper-lower value)	n	Median (upper-lower value)	n	Median (upper-lower value)	
Cells/mL	51	19.1 (6.7-75.6)	32	16.9 (3.5-44.0)	71	21.8 (5.6-68.0)	8	17.8 (11.6-28.6)	NS
Lymphocytes (%)	100	25.2 (0.0-70.3)	58	31.3 (1.0-73.0)	114	30.0 (1.6-95.1)	15	29.8 (7.7-48.8)	NS
Neutrophils (%)	100	1.0 (0.0-15.2)	58	1.0 (0.0-30.2)	114	1.1(0.0-34.9)	15	1.4 (0.2-4.2)	NS
CD4+ (%)	82	80.0 (39.0-93.0)	38	74.1 (47.0-93.0)	86	74.5 (28.0-94.0)	14	85.5 (64.0-91.0)	[A]0.010 [B]0.001 [C]0.003 [D]0.004
CD8+ (%)	82	12.0 (2.0-50.0)	38	17.9 (3.0-41.0)	86	17.0 (4.0-62.0)	14	9.5 (3.0-28.0)	[A]0.005 [B]0.002 [C]0.003 [D]0.002
CD4+/CD8+ ratio	82	6.6 (0.8-39.0)	38	4.3 (1.3-31.0)	87	4.5 (0.5-22.0)	14	8.9 (2.4-30.0)	[A]0.004 [B]0.001 [C]0.003 [D]0.002
CD103+CD4+/CD4+ ratio	34	0.050 (0.00-0.52)	21	0.10 (0.01-0.44)	49	0.090 (0.01-0.62)	7	0.040 (0.00-0.61)	[B]0.024
PB CD4+/CD8 + ratio	76	2.2 (0.4-15.5)	37	1.8 (0.0-9.0)	82	1.62 (0.60-16.3)	14	1.6 (0.6-3.4)	[A]0.005 [B]0.003

*03 and *15 were typed using tag SNPs.
Median (upper-lower value)
LS:Löfgren's syndrome, non-LS:non-Löfgren's syndrome, PB:Peripheral Blood
[A] =*03+/*15- versus *03-/*15+,
[B]= *03+/*15- versus *03-/*15-,
[C]= *03+/*15+ versus *03-/*15-,
[D] =*03-/*15+ versus *03+/*15+

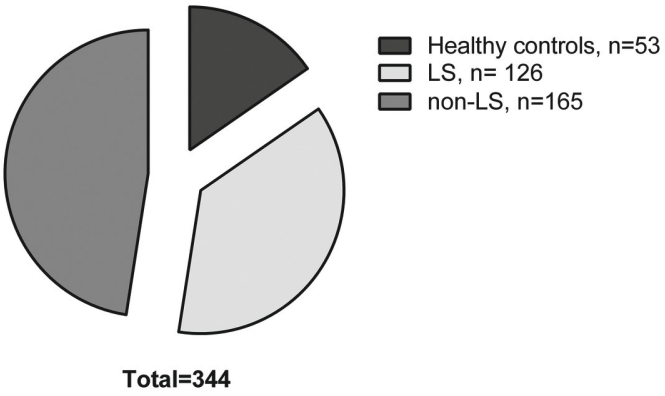


Figure 1. Overview of patients and healthy controls.
LS= Löfgren's syndrome; non-LS= non-Löfgren's syndrome.

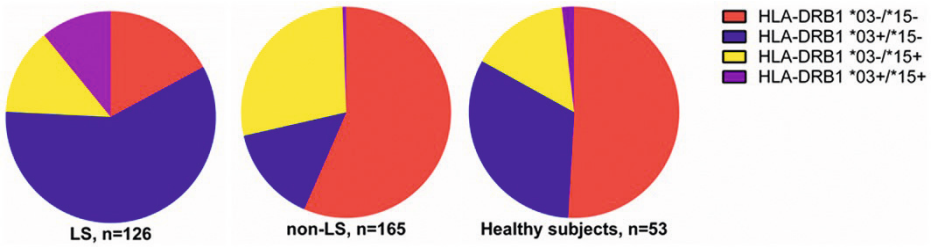


Figure 2. Human leukocyte antigen (HLA) genotypes in Löfgren's syndrome (LS), non-LS and healthy subjects, respectively.

In all patients (LS and non-LS), no significant differences were found in % of BAL lymphocytes and % of neutrophils between carriers and non-carriers. Significant differences were observed in % of lymphocyte subsets and ratios (figure 3a). The highest BAL CD4+/CD8+ ratio was found in *03+/*15+ patients (median ratio = 8.9; 2.36-30.00) (figure 3b). We found no statistical significant difference between *03+/*15+ and *03+/*15-. The BAL CD4+/CD8+ ratio in *03+/*15- patients (median ratio = 6.6; 0.78-39.00) was significantly higher compared to *03-/*15+ patients (p=0.004) and *03-/*15- patients (p=0.001). Patients with *03-/*15+ had the lowest BAL CD4+/CD8+ratio (median ratio = 4.26; 1.29-31.00). The median CD103+CD4+/CD4+ ratio was decreased (reference value<0.20[18]) in all groups (figure 3c). Patients with *03+/*15- had a higher CD103+CD4+/CD4+ ratio compared to *03-/*15- (p=0.024).

The CD4+/CD8+ ratio in peripheral blood was highest in the *03+/*15- patients (median ratio = 2.2; 0.38-15.50), which was significantly higher compared to *03-/*15+ patients (p=0.005) and *03-/*15- patients (p=0.003) (figure 3d).

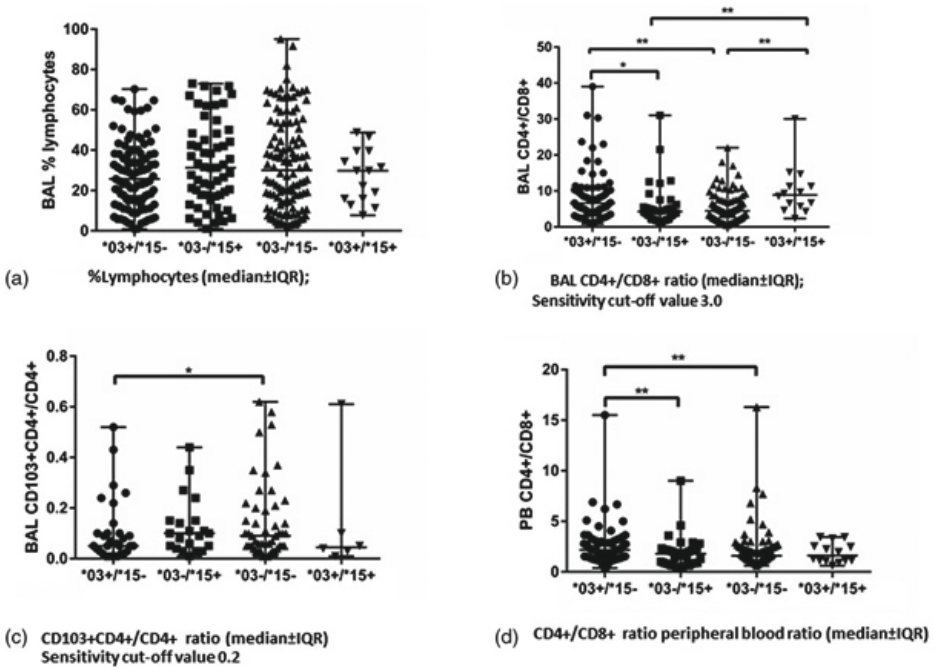


Figure 3. Bronchoalveolar lavage (BAL) cell percentage of lymphocytes and cell phenotype ratios in all patients stratified per carriership of DRB1*03 and *15 tag. *$P \leq 0{\cdot}05$; **$P \leq 0{\cdot}01$. (a) %Lymphocytes [median ± interquartile range (IQR)]; (b) BAL $CD4^+/CD8^+$ ratio (median ± IQR) Sensitivity cut-off value 3·0[18]; (c) $CD103^+CD4^+/CD4^+$ ratio (median ± IQR) Sensitivity cut-off value 0·2[18]; (d) $CD4^+/CD8^+$ ratio (median ± IQR) peripheral blood.

We performed a subanalysis to determine the influence of smoking on the BAL outcomes. Comparable results were demonstrated in non-smokers; however, in smokers only the BAL CD4+/CD8+ ratio was significantly higher in *03+/*15- (median ratio = 6.0; 2.7-30.3) compared to *03-/*15+ (median ratio = 1.84; 1.62-2.06), p=0.022. In addition, no significant differences were found between different HLA genotypes in smokers in BAL CD103+CD4+/CD4+ ratio and PB CD4+/CD8+ ratio.

The group of patients using oral corticosteroids was too small to perform a sub analysis, therefore we excluded these patients. In this group (without patients using oral corticosteroids) the results of BAL outcomes in four different HLA-DRB1 genotypes were comparable to the whole group.

Bronchoalveolar lavage outcomes: Löfgren's syndrome versus non-Löfgren's syndrome

Results from BAL analysis of patients with LS are shown in table 4 and figure 4. LS patients with *03+/*15- showed a significantly lower percentage of lymphocytes (median = 23.8;

0.0-70.3), compared to *03-/*15+ patients, who showed the highest percentage of lymphocytes (median = 42.0; 11.3-71.8; p=0.011) (figure 4a). Patients with *03+/*15- and *03+/*15+ showed a higher CD4+/CD8+ ratio in lavages, a higher CD4+/CD8+ ratio in PB and a lower CD103+CD4+/CD4+ ratio in lavages compared to *03-/*15+ and *03-/*15- patients, but these differences were statistically not significant (figure 4b-d).

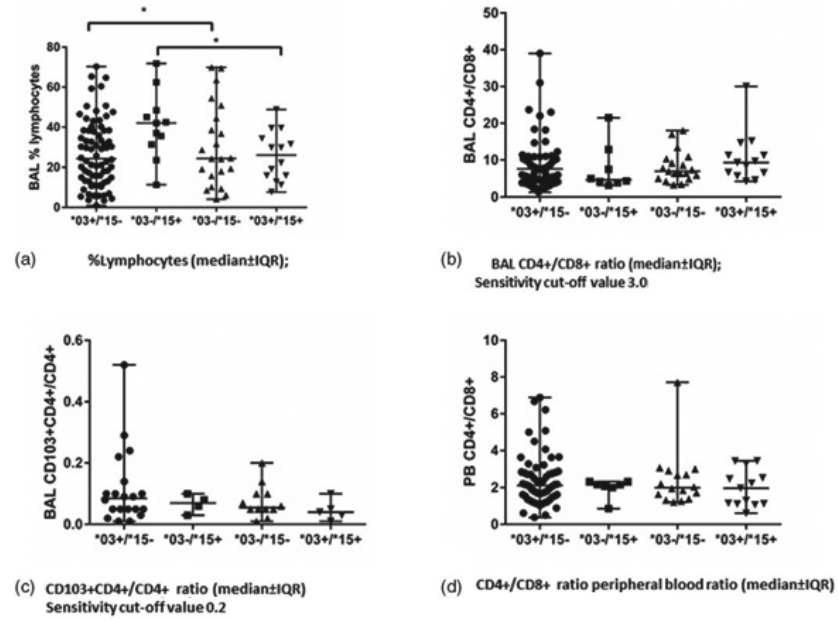


Figure 4. Bronchoalveolar lavage (BAL) cell percentage of lymphocytes and cell phenotype ratios in Löfgren's syndrome (LS) stratified per carriership of DRB1*03 and *15 tag. *P ≤ 0·05. (a) %Lymphocytes [median ± interquartile range (IQR)]; (b) BAL $CD4^+/CD8^+$ ratio (median ± IQR) Sensitivity cut-off value 3·0[18]; (c) $CD103^+CD4^+/CD4^+$ ratio (median ± IQR) Sensitivity cut-off value 0·2[18]; (d) $CD4^+/CD8^+$ ratio (median ± IQR) peripheral blood.

BAL outcomes of non-LS patients comparing distinct genotypes are shown in table 4 and figure 5. No significant differences were found when comparing the percentages of lymphocytes or neutrophils in the BAL of different *HLA-DRB1* genotypes in non-LS patients.

Patients with*03+/*15- had a lower CD103+CD4+/CD4+ ratio (median ratio = 0.030; 0.01-0.43) than *03-/*15+ patients (median ratio 0.11; 0.01-0.44) and *03-/*15- patients (median ratio = 0.10; 0.01-0.62), p=0.045 and p=0.018 respectively (figure 5c). Furthermore, the CD4+/CD8+ ratio in the peripheral blood of *03+/*15- patients was higher (median ratio = 2.6, 1.3-15.5), than in the *03-/*15- patients (median ratio 1.5; 0.6-16.3; p=0.011) (figure 5d).

Table 4. Bronchoalveolar lavage and peripheral blood (PB) findings of LS and non-LS categorized in four *HLA-DRB1* genotypes: *03+/*15-, *03-/*15+, *03-/*15-, *03+/*15+

	*03+/*15-		*03-/*15+		*03- /*15-		*03+ / *15+		P
LS	n	Median (upper-lower value)	n	Median (upper-lower value)	n	Median (upper-lower value)	n	Median (upper-lower value)	
Cells/mL	39	18.5 (7.5-75.6)	4	27.8 (12.0-30.7)	13	23.7 (7.5-53.9)	7	16.9 (11.6-24.0)	NS
Lymphocytes (%)	75	23.8 (0.0-70.3)	11	42.0 (11.3-71.8)	22	24.4 (4.1-69.9)	14	26.0 (7.7-48.8)	[A]0.011 [B]0.020
Neutrophils (%)	75	1.1 (0.0-15.2)	11	0.9 (0.0-4.70)	22	1.2(0.0-31.2)	14	1.4 (0.2-4.2)	NS
CD4+ (%)	67	81.0 (47.0-93.0)	8	79.5 (73.0-90.0)	18	82.0 (56.0-94.0)	13	86.0 (64.0-91.0)	NS
CD8+ (%)	67	11.0 (2.0 36.0)	8	16.9 (4.0-23.0)	18	12.0 (5.0-22.0)	13	9.0 (3.0-16.0)	NS
CD4+/CD8+ ratio	67	7.6 (1.3-39.0)	8	4.7 (3.2-21.5)	19	7.0 (3.3-18.0)	13	9.3 (4.3-30.0)	NS
CD103+CD4+/CD4+ ratio	23	0.05 (0.0-0.5)	4	0.07 (0.03-0.10)	12	0.06 (0.01-0.20)	6	0.04 (0.00-0.10)	NS
PB CD4+/CD8+ ratio	62	2.1 (0.4-6.9)	8	2.1 (0.00-2.3)	16	2.0 (1.2-7.7)	13	2.0 (0.6-3.4)	NS
Non-LS*	n	Median (upper-lower value)	n	Median (upper-lower value)	n	Median(upper-lower value)			
Cells/mL	12	21.3 (6.7-54.4)	28	16.7 (3.5-44.0)	58	21.6 (5.6-68.0)			NS
Lymphocytes (%)	25	27.6 (1.1-64.5)	47	26.5 (1.0-73.0)	92	32.8 (1.6-95.1)			NS
Neutrophils (%)	25	0.8 (0.10-9.20)	47	1.0 (0.1-30.2)	92	1.1 (0.0-34.9)			NS
CD4+ (%)	15	71.0 (39.0-93.0)	30	73.5 (47.0-93.0)	68	74.0 (28.0-93.0)			NS
CD8+ (%)	15	17.0 (3.0-50.0)	30	19.5 (3.0-41.0)	68	20.0 (4.0-62.0)			NS
CD4+/CD8+ ratio	15	3.5 (0.78-30.3)	30	3.8 (1.3-31.0)	68	3.8 (0.5-22.0)			NS
CD103+CD4+/CD4+	11	0.03 (0.01-0.43)	17	0.11 (0.01-0.44)	37	0.1 (0.01-0.62)			[A]0.045 [C]0.018
PB CD4+/CD8+ ratio	14	2.6 (1.3-15.5)	29	1.5 (0.3-9.0)	66	1.5 (0.6-16.3)			[A]0.019 [C]0.011

*03 and *15 were typed using tag SNPs. Data is expressed as median (upper-lower value)
LS: Löfgren's syndrome, non-LS: non-Löfgren's syndrome, PB: Peripheral Blood
*Our cohort included only one *03+/*15+ non-LS patients, therefore this group was to small and was excluded from analysis
[A] =*03+/*15- versus *03-/*15+,
[B] = *03-/*15+ versus *03+/*15+
[C]= *03+/*15- versus *03-/*15-

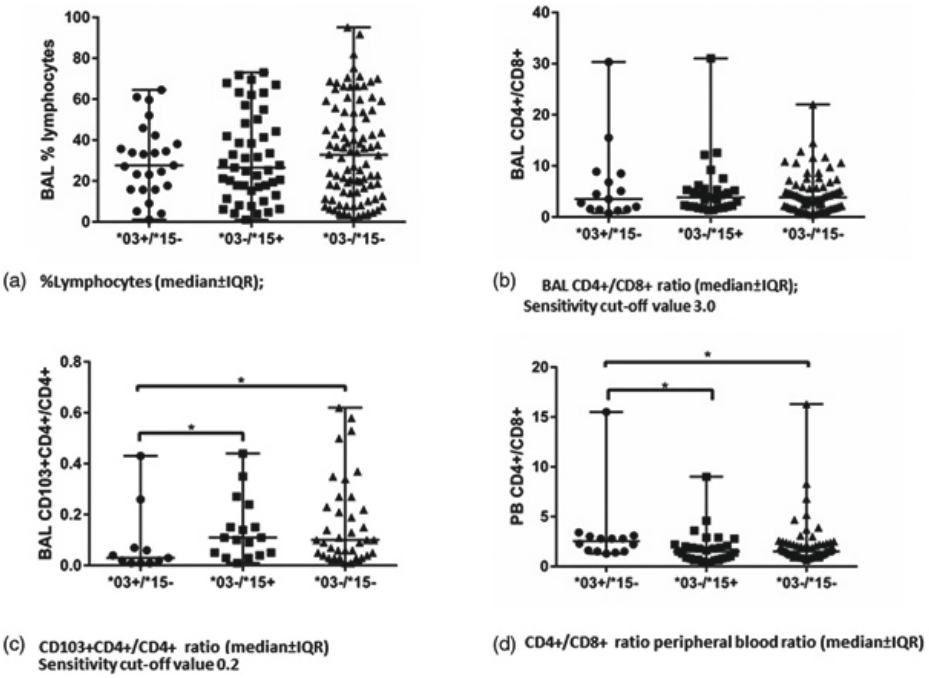


Figure 5. Bronchoalveolar lavage (BAL) cell percentage of lymphocytes and cell phenotype ratios [median ± interquartile range (IQR)] in non-Löfgren's syndrome (LS) stratified per carriership of DRB1*03 and *15 tag. *P ≤ 0·05. (a) %Lymphocytes [median ± interquartile range (IQR)]; (b) BAL $CD4^+/CD8^+$ ratio (median ± IQR) Sensitivity cut-off value 3·0[18]; (c) $CD103^+CD4^+/CD4^+$ ratio (median ± IQR) Sensitivity cut-off value 0·2[18]; (d) $CD4^+/CD8^+$ ratio (median ± IQR) peripheral blood.

DISCUSSION

Most sarcoidosis patients have a good prognosis, although a clear proportion of patients develop a chronic and/or progressive disease. It is a clinical challenge to distinguish patients who will develop a chronic course, from patients who are more likely to spontaneously resolve disease. Usually the only way is to monitor all patients on a regular basis to identify the few who develop chronic disease. Detailed analysis of BAL in combination with *HLA-DRB1**03 and *15 typing may aid to early identification of these patients. We studied whether clinically informative HLA types associate with BAL cell characteristics.

With respect to the goals of our study, we confirmed the association of *HLA-DRB1**03 with the A allele of rs2040410 and -*DRB1**15 with the A allele of rs3135388[15]. The SNPs tagging *HLA-DRB1**03 and *HLA-DRB1**15 have each been used before independently in other diseases, diabetes and multiple sclerosis, respectively[26,27]. To our knowledge, this

is the first study that validated a tag SNP with HLA typing in sarcoidosis patients. Several studies have shown that *HLA-DRB1**03 positive LS patients have a favourable prognosis, with recovery within 2 years in 95% of the cases. In contrast, only half of the *HLA-DRB1**03 negative LS patients experience a resolving course of disease. Furthermore, more than half of the non-resolving *HLA-DRB1**03 patients are *HLA-DRB1**15-positive[6]. This association of *HLA-DRB1**15 with chronic disease has been confirmed by others[10,28]. Due to its complexity, complete HLA typing is not very suitable for daily clinical practice. However, the use of SNP tagging is simple and affordable, and allows easy identification of patients with a good prognosis and patients who will develop a chronic course of disease.

Over decades various studies have investigated the characteristics of BAL and its relation to prognosis of disease in different phenotypes of sarcoidosis. Drent et al. demonstrated that patients with an acute presentation of sarcoidosis with arthritis and erythema nodosum had significantly higher proportions of lymphocytes and CD4+/CD8+ ratio than patients with respiratory and general constitutional symptoms[20]. This study and other studies implied a possible beneficial role of CD4+ T-lymphocytes[29,30]. In the whole group, the CD4+/CD8+ ratio was higher in *03+ patients compared to *03- patients in our combined cohort. In LS patients a similar difference was observed, which did not reach statistical significance, due probably to the small group of patients in the analyses. In our LS cohort higher lymphocyte percentage was found in the patients with *03+/*15- versus *03-/*15+. A few papers described BAL characteristics comparing *HLA-DRB1**03-positive and -negative sarcoidosis patients. Idali et al.[31] did not observe differences in the BAL lymphocyte percentage between *HLA-DRB1**03-positive and -negative patients. However, they did not make a distinction between LS and non-LS patients, nor did they include other HLA-genotypes than *HLA-DRB1**03. In line with their findings, in our combined cohort there are also no differences in lymphocyte percentages between *03+ and *03-patients. A striking finding is that in LS patients the *03-/*15+ patients had significantly higher lymphocyte percentages compared to *03+/*15- patients.

In a cohort of 118 sarcoidosis patients, Planck et al.[22] also observed a decreased lymphocyte percentage and an increased CD4+/CD8+ratio in BAL from *HLA-DRB1**03-positive patients compared to those negative for *HLA-DRB1**03. Although their cohort included 43% LS patients, LS and non-LS patients were not analysed separately, nor was the contribution of the *HLA-DRB1**15 allele studied. Similar findings regarding lymphocyte percentage and CD4+/CD8+ ratio in *HLA-DRB1**03-positive patients were reported in a more recent study that included LS patients[32]. Unfortunately, other

HLA genotypes were not studied. Our results show that besides a low lymphocyte percentage, a high CD4+/CD8+ ratio provides a good prognosis.

In addition, a more recent paper published by Kinloch et al. found lower lymphocyte counts in combination with a higher CD4+/CD8+ ratio in BAL fluid of *HLA-DRB1**03 negative patients[21]. However, none of the above mentioned studies had investigated the CD4+/CD8+ ratio in the peripheral blood. In our cohort a total of 15 patients were positive for both *03 and *15. The effect of carriage of *HLA-DRB1**15 in *-DRB1**03+ patients is not known, one allele could be dominant over the other or the combined carriage could cancel the effect. Due to low patient number in this group no hard conclusions can be made. However, a significantly higher percentage of lymphocytes was found in *03-/*15+ patients compared to *03+/*15+ patients. This finding suggests that the influence of *03 is dominant over the influence over *15 in patients with Löfgren's syndrome. In essence, this is implicated by the studies from Grunewald, and is therefore most likely[12,22].

Braun et al., who analysed CD4+ T cells from the BAL of a variety of fibrotic lung diseases, suggested that CD103+ cells are terminally differentiated effector T cells that might be involved in the process of lung fibrosis[33]. Several authors have reported that patients with a more advanced radiologic stage of sarcoidosis show a higher proportion of CD4+ T lymphocytes expressing CD103 and having a higher CD103+CD4+/CD4+ ratio[18,19]. Our current findings support this by showing that sarcoidosis patients with *03+/*15-, who are generally known as having a favourable prognosis, have a decreased CD103+CD4+/CD4+ ratio compared with *03-/15+ and compared with *03-/*15- patients. Our data suggest that a decreased CD103+CD4+/CD4+ ratio predict for a benign course of disease. Sarcoidosis has traditionally been regarded as a Th1-driven disease, characterised by excessive interferon (IFN)-γ, interleukin (IL)-12, and tumour necrosis factor (TNF)-α production in the lungs[34]. By separating patients into LS and non-LS or stratifying by HLA type, differences in effector T-cell subsets were seen by Moller and co-workers[34]. Our data show that the type of lymphocytes is important in redirecting the inflammation towards a self-limiting or chronic disease. Further studies need to be conducted to elucidate the complex interactions between genetics, T cell function and clinical behaviour of the disease.

Whether an inflammatory immune response results in a self-limiting or a chronic relapse-remitting type of disease is dependent on multiple factors. Important factors are type and quantity of the antigens, the extent of antigen presentation in terms of tissue and duration, the context of antigen presentation, and genetic composition. The net balance determines whether the CD4 response is balanced with a beneficial CD4 T regulatory component, or dominated by pro-inflammatory Th17.1 cells.

Regarding genetic composition, DR3 is generally a good prognostic factor for LS, irrespective of the second DR allele. Detailed immunologic analysis of LS cohorts revealed that Vα2.3+Vβ22+ CD4+ T-cells are expanded in these patients[32]. Their phenotype and cytokine profiles in lavages of LS patients demonstrate profiles to be less skewed towards proinflammatory Th17.1 cells[35], and it is speculated these cells may recognise autoantigens such as vimentin[21]. Current manuscript points out that DR3 positivity does not guarantee an inflammatory process to become self-limiting, and a proportion of the patients develop sarcoidosis. Considering the lower CD4+/CD8+ ratios particularly observed in sarcoidosis patients, the capacity to establish a more Th1-like response and involve CD8+ T cells in the inflammatory response may mark a relapse-remitting type of response. Whether the DR3 non-LS patients are incapable of producing Vα2.3+Vβ22+ CD4 T cells, or vimentin is not involved in the inflammatory response, remains to be determined. Furthermore, studies aiming to identify the triggers in sarcoidosis[36] will help to identify the Th phenotype and establish their role in the immunological puzzle that results in a self-limiting or a relapse-remitting type of disease, along with DR type.

Due to its retrospective design, not all parameters were available for all patients. Furthermore, 23% of the patients were current smokers. Smoking increases the total cell count and reduces the lymphocyte percentage and CD4+/CD8+ ratio in BAL[20]. However, the percentage of smokers was similar in both groups, LS and non-LS. Also, the duration between BAL and diagnosis in LS patients, a maximum of 4 months, could have possibly influenced the BAL outcomes, because LS is known for its acute inflammation. The strength of our study is that for confirmation of tag SNPs full *HLA-DRB1**typing was performed in a laboratory that regularly participates in external proficiency testing in order to ensure the quality of the laboratory. Furthermore, we separated the patients in four groups (*03+/*15-, *03-/*15+, *03-/*15-, *03+/*15+), in order to study the influence of both clinical course and associated tag SNPs.

In conclusion, in this study we show that *HLA-DRB1**03 and *DRB1**15 can be perfectly approximated by genotyping of tag SNPs, which can be used easily in daily clinical practice to distinguish between patients who will develop a chronic course or not. Secondly, we found a significantly higher CD4+/CD8+ ratio in *03+/*15- patients in the whole group. We also found a lower lymphocyte percentage in *03+/*15- LS patients, and a decreased CD103+CD4+/CD4+ ratio*03+/*15- non-LS patients. Our results indicate that a phenotype and HLA-markers of favourable disease associate with a low lymphocyte percentage, high CD4+/CD8+ ratio and low CD103+CD4+/CD4+ ratio in BAL.

REFERENCES

1. Löfgren, S. & Lundbäck, H. The bilateral hilar lymphoma syndrome; a study of the relation to age and sex in 212 cases - PubMed. *Acta Med Scand.* **142**, 265–73 (1952).
2. Löfgren, S. & Lundbäck, H. The Bilateral Hilar Lymphoma Syndrome. A Study of the Relation to Tuberculosis and Sarcoidosis in 212 Cases. *Acta Med. Scand.* **142**, 265–273 (1952).
3. Löfgren, S. Primary Pulmonary Sarcoidosis. I. Early Signs and Symptoms. *Acta Med. Scand.* **145**, 424–431 (1953).
4. Löfgren, S. Primary Pulmonary Sarcoidosis. II. Clinical Course and Prognosis. *Acta Med. Scand.* **145**, 465–474 (1953).
5. Karakaya, B., Kaiser, Y., van Moorsel, C. H. M. & Grunewald, J. Lofgren's Syndrome: Diagnosis, Management, and Disease Pathogenesis. *Semin. Respir. Crit. Care Med.* **38**, 463–476 (2017).
6. Grunewald, J. & Eklund, A. Löfgren's syndrome: Human leukocyte antigen strongly influences the disease course. *Am. J. Respir. Crit. Care Med.* **179**, 307–312 (2009).
7. Hedfors, E. & Lindström, F. HLA-B8/DR3 in sarcoidosis: Correlation to acute onset disease with arthritis. *Tissue Antigens* **22**, 200–203 (1983).
8. Smith, M. I. *et al.* Association of HLA B8 with spontaneous resolution in sarcoidosis. *Thorax* **36**, 296–298 (1981).
9. Sato, H. *et al.* HLA-DQB1*0201: A marker for good prognosis in British and Dutch patients with sarcoidosis. *Am. J. Respir. Cell Mol. Biol.* **27**, 406–412 (2002).
10. 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* **156**, 1601–1605 (1997).
11. 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.* **169**, 696–702 (2004).
12. Grunewald, J. *et al.* Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. *Respir. Res.* **11**, 25 (2010).
13. Rossman, M. D. *et al.* HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am. J. Hum. Genet.* **73**, 720–735 (2003).
14. Rivera, N. V *et al.* High-Density Genetic Mapping Identifies New Susceptibility Variants in Sarcoidosis Phenotypes and Shows Genomic-driven Phenotypic Differences. *Am. J. Respir. Crit. Care Med.* **193**, 1008–1022 (2016).
15. De Bakker, P. I. W. *et al.* A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat. Genet.* **38**, 1166–1172 (2006).
16. Drent, M., Mansour, K. & Linssen, C. Bronchoalveolar lavage in sarcoidosis. *Semin. Respir. Crit. Care Med.* **28**, 486–495 (2007).
17. Winterbauer, R. H. *et al.* Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. *Chest* **104**, 352–361 (1993).
18. Heron, M. *et al.* Evaluation of CD103 as a cellular marker for the diagnosis of pulmonary sarcoidosis. *Clin Immunol.* **126**, 338–44 (2008).

19. Mota, P. C. *et al.* Diagnostic value of CD103 expression in bronchoalveolar lymphocytes in sarcoidosis. *Respir. Med.* **106**, 1014–1020 (2012).

20. Drent, M., Van Velzen-Blad, H., Diamant, M., Hoogsteden, H. C. & Van den Bosch, J. M. M. Relationship between presentation of sarcoidosis and T lymphocyte profile; A study in bronchoalveolar lavage fluid. *Chest* **104**, 795–800 (1993).

21. Kinloch, A. J. *et al.* In Situ Humoral Immunity to Vimentin in HLA-DRB1*03+ Patients With Pulmonary Sarcoidosis. *Front. Immunol.* **9**, 1516 (2018).

22. Planck, A., Eklund, A. & Grunewald, J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **18**, 64–9 (2001).

23. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **16**, 149–173 (1999).

24. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Report of the European Society of Pneumology Task Group - PubMed. *Eur Respir J* **2**, 561–585 (1989).

25. Haslam, P. & Baughman, R. P. Report of ERS Task Force: guidelines for measurement of acellular components and standardization of BAL. *Eur. Respir. J.* **14**, 245–248 (1999).

26. Barker, J. M. *et al.* Two single nucleotide polymorphisms identify the highest-risk diabetes HLA genotype: potential for rapid screening. *Diabetes* **57**, 3152–3155 (2008).

27. Hafler, D. A. *et al.* Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* **357**, 851–862 (2007).

28. Voorter, C. E. M. *et al.* HLA class II amino acid epitopes as susceptibility markers of sarcoidosis. *Tissue Antigens* **70**, 18–27 (2007).

29. Doubková, M., Pospíšil, Z., Skřičková, J. & Doubek, M. Prognostic markers of sarcoidosis: An analysis of patients from everyday pneumological practice. *Clin. Respir. J.* **9**, 443–449 (2015).

30. Prasse, A. *et al.* Phenotyping sarcoidosis from a pulmonary perspective. *Am. J. Respir. Crit. Care Med.* **177**, 330–336 (2008).

31. Idali, F. *et al.* Reduced Th1 response in the lungs of HLA-DRB1*0301 patients with pulmonary sarcoidosis. *Eur. Respir. J.* **27**, 451–459 (2006).

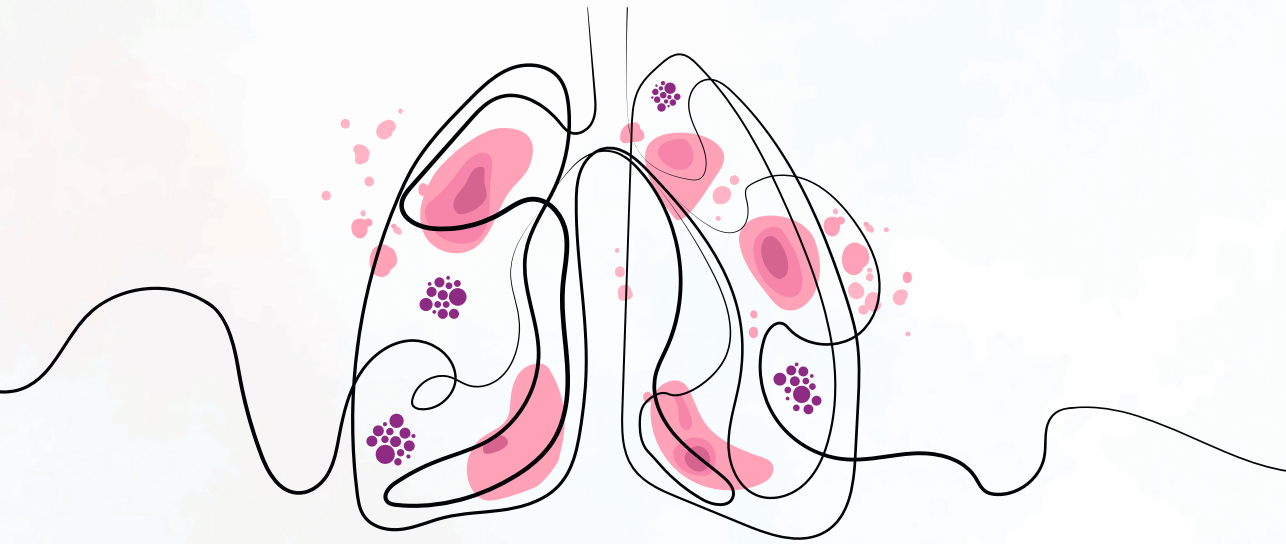
32. Grunewald, J. *et al.* T-cell receptor-HLA-DRB1 associations suggest specific antigens in pulmonary sarcoidosis. *Eur. Respir. J.* **47**, 898–909 (2016).

33. Braun, R. K. *et al.* Phenotypic and molecular characterization of CD103+ CD4+ T cells in bronchoalveolar lavage from patients with interstitial lung diseases. *Cytometry B. Clin. Cytom.* **54**, 19–27 (2003).

34. Moller, D. R. *et al.* Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. *J Immunol.* **156**, 4952–60 (1996).

35. Kaiser, Y. *et al.* Expanded lung T-bet+RORγT+ CD4+ T-cells in sarcoidosis patients with a favourable disease phenotype. *Eur. Respir. J.* **48**, 484–494 (2016).

36. Beijer, E., Veltkamp, M., Meek, B. & Moller, D. R. Etiology and Immunopathogenesis of Sarcoidosis: Novel Insights. *Semin. Respir. Crit. Care Med.* **38**, 404–416 (2017).



CHAPTER 5

QUANTIFICATION OF PULMONARY DISEASE ACTIVITY IN SARCOIDOSIS MEASURED WITH ^{18}F-FDG PET/CT: SUVMAX VERSUS TOTAL LUNG GLYCOLYSIS

M.C. Schimmelpennink, A.D.M. Vorselaars, M. Veltkamp, R.G.M. Keijsers

EJNMMI Res. 2019 Jun 13;9(1):54.

ABSTRACT

BACKGROUND

^{18}F-FDG PET/CT has proven to be a reliable tool for therapy monitoring in sarcoidosis. Previous PET studies investigated the maximum standardised uptake value (SUVmax) as marker for disease activity. Total lung glycolysis (TLuG) is a new tool, quantifying the glycolysis of the entire lung. Since SUVmax represents the maximum activity in only one pixel, we hypothesise that TLuG is a more accurate marker for active pulmonary disease and predictor of response than SUVmax.

METHODS

In this retrospective cohort study 27 patients started on infliximab for refractory pulmonary sarcoidosis. Patients received infliximab intravenously monthly at a dose of 5 mg/kg. We performed a lung function test and a ^{18}F-FDG PET/CT before initiation of infliximab and after 6 months of treatment. SUVmax and TLuG were determined of the pre- and post-scan. Change in lung function was correlated with the change in SUVmax and TLuG, and was correlated to the initial SUVmax and TLuG to evaluate the predictive value of initial metabolic activity.

RESULTS

Change in SUVmax (ΔSUVmax) significantly correlated with change in forced vital capacity (ΔFVC) ($r=-0.497$, $p=0.008$) and with change in forced expiratory volume in one second (ΔFEV1) ($r=-0.467$, $p=0.014$). Furthermore, change in TLuG (ΔTLuG) significantly correlated with ΔFVC($r=-0.430$, $p=0.025$), ΔFEV1 ($r=-0.532$, $p=0.004$) and change in diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (ΔDLCOc) ($r=-0.423$, $p=0.039$). ΔSUVmax and ΔTLuG significantly correlated ($r= 0.735$, $p<0.001$). Initial SUVmax significantly correlated with ΔFVC, and ΔDLCOc. In addition, initial TLuG significantly correlated with ΔFEV1 and ΔDLCOc. A SUVmax>7.5 at initiation of infliximab was predictive for 5% response in FVC, whereas SUVmax>9.2 was predictive for 5% response in DLCOc. In addition, high TLuG>4100 at initiation of infliximab was predictive for 5% response in FVC and FEV1 and TLuG>4500 was predictive for response in DLCOc.

CONCLUSION

SUVmax and TLuG are equal in determining the response to infliximab in pulmonary sarcoidosis patients. Furthermore, SUVmax and TLuG at initiation of infliximab can predict change in lung function after treatment. Since TLuG is a more time-consuming tool, we recommend to use SUVmax of the lung parenchyma for response monitoring in pulmonary sarcoidosis.

INTRODUCTION

Sarcoidosis is a granulomatous multisystemic disease with both a heterogeneous presentation and clinical course[1]. Several biomarkers are determined in the standard diagnostic work-up and follow-up of patients with sarcoidosis, like serum angiotensin converting enzyme (ACE) and soluble interleukin 2 receptor (sIL-2R) in serum, as well as lymphocytes and CD4+/CD8+ ratio in bronchoalveolar lavage[2–4]. ^{18}F-Fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) has proven to be a reliable biomarker to measure disease activity in sarcoidosis[5,6] and to detect occult sarcoidosis lesions[7,8]. Maximum standardised uptake value (SUVmax) is the most commonly used semi-quantitative value of ^{18}F-FDG PT/CT in sarcoidosis. In clinical oncology, total lesion glycolysis can additionally be used to quantify activity on ^{18}F-FDG PET/CT. Total lesion glycolysis is measured as the product of the SUVmean and the metabolic volume of the lesion. Total lesion glycolysis is used in the standard follow-up in patients with malignancies for response rating after treatment[9]. Furthermore, total lesion glycolysis has proven to be a better prognostic marker than SUVmax in patients with malignancies[10]. As SUVmax is only derived from activity in one pixel, it is insufficient to objectify the global inflammation of the lungs. Total lung glycolysis is a new tool, that is a derivative of the total lesion glycolysis focused on the lungs. To our knowledge, no studies have been performed investigating the response rate of sarcoidosis patients to infliximab using the semi-quantitative total glycolysis of the lung (TLuG). We hypothesise that determining the amount of inflammatory activity in pulmonary sarcoidosis will be more accurate by using TLuG than by SUVmax. The aim of our study is to compare the prognostic value of SUVmax and TLuG regarding the change in lung function in pulmonary sarcoidosis patients treated with infliximab.

METHODS

Study population

This study is a retrospective cohort study consisting of 27 patients with refractory pulmonary sarcoidosis indicated for infliximab treatment. All consecutive patients started infliximab therapy between July 2010 and September 2015. Sarcoidosis was defined as refractory when organ damage persisted while receiving second-line immunosuppressive treatment or when second-line therapy had to be discontinued due to toxicity.

All patients received infliximab at a dose of 5mg/kg intravenously at week 0, week two and thereafter every four weeks. Lung function and ^{18}F-FDG PET/CT were routinely

performed before and after the induction phase of 26 weeks. Sarcoidosis was diagnosed according to the guidelines of American Thoracic Society/European Respiratory Society/ World Association of Sarcoidosis and other Granulomatous Disorders (ATS/ERS/ WASOG) statement[11]. The following data were extracted from patient records: gender, race, smoking history, organ involvement and Scadding stage. The study was approved by the investigational review board of St. Antonius Hospital Nieuwegein (registration number LTME/Z-12.033 and acronym ORATS).

Lung function

Lung function was performed before and six months after induction of infliximab. Lung function tests were performed using Master Screen Body (Jaeger ms-pft analyse unit, Würzberg, Germany). Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCOc) were expressed as percentages of predicted. Furthermore, the change of the pulmonary function parameters after 26 weeks of infliximab treatment were measured and expressed as ΔFVC, ΔFEV1 and ΔDLCOc.

^{18}F-FDG PET/CT

^{18}F-FDG PET/CT was performed in accordance with the joint guideline of the Society of Nuclear Medicine and European Association of Nuclear Medicine[12]. FDG PET/CT was performed with a Philips Gemini Time of Flight PET/CT scanner (Philips Medical Systems, Best, the Netherlands). The department of Nuclear Medicine of the St. Antonius Hospital is an EARL accredited PET/CT center. Low-dose CT was used for attenuation correction and optimizing image interpretation. Reconstruction of the PET images is performed in accordance with the 3D–row action maximum likelihood algorithm protocol (RAMLA), applying 4 iterations with a 144 × 144 matrix. A quadratic FDG dosage regimen was used based on the patient's body weight with a minimum of 37 Mega Becquerel (MBq) and a maximum of 400 MBq. Emission scan was performed from the subinguinal region to the head. The SUVmax was determined by two observers (RK and MS). The SUVmax was calculated in the lung parenchyma as described before[13]. Region of interest (ROI) was 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 after infliximab. ROI drawing was performed using the automatic ROI drawing tool in the Hermes Diagnostics program (Hermes Medical Solutions, Stockholm, Sweden). TLuG, the total lung glycolysis, is a derivative of the Total Lesion Glycolysis (TLG). In contrast with TLG, focusing on a lesion, TLuG is focused on an organ, i.e. the lung. The TLuG provides information regarding the cumulative metabolic activity in the lung parenchyma, as described previously in the paper of Adams et al[14]. TLuG was determined by two nuclear medicine physicians (RK and HA). The lung parenchyma is therefore our volume of

interest (VOI), Figure 1. This VOI was determined semi-automatically by CT based on Hounsfield Units (HU) in accordance with Adams et al[14]. VOI was measured by using a lung segmentation program provided by Hermes Medical Solutions (Stockholm, Sweden). This CT based VOI served as a demarcated volume in PET in which the total metabolic activity was measured, expressed as TLuG, SUVmean and SUVmax.

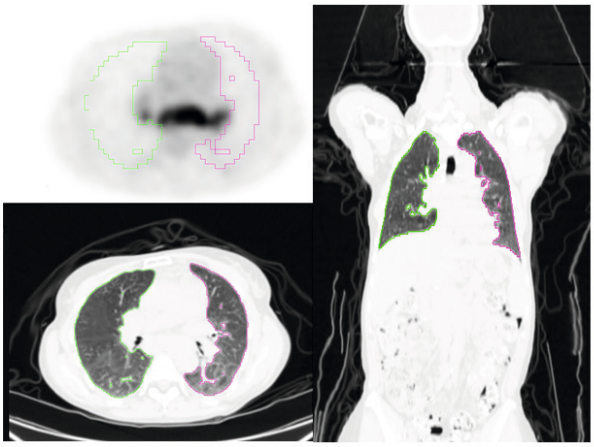


Figure 1. Example of VOI of total lung glycolysis. TLuG is the cumulative metabolic activity in the total lung parenchyma. Additionally, SUVmax and SUVmean are determined in the VOI.

Statistics

All analyses were performed using IBM SPSS Statistics 24. Continuous variables were expressed as mean ± standard deviation. Changes between baseline outcomes and outcomes after treatment with infliximab were analysed with the two-tailed paired t-test. Correlation between the change in lung function (ΔFVC, ΔFEV1, ΔDLCOc) and change in SUVmax (ΔSUVmax) and TLuG (ΔTLuG) were measured by the Pearson correlation coefficient. Pearson correlation coefficient (expressed as r) of 0.9-1.0 was considered as a very high correlation, 0.7-0.9 high correlation, 0.5-0.7 moderate, 0.3-0.5 low, 0.0-0.3 negligible[15]. The interobserver variability for TLuG was measured with the intraclass correlation coefficient. The optimal cut-off point of SUVmax and TLuG to predict 5% response in lung function (5% FVC, FEV1, DLCOc % of predicted) was found by maximising the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. Cut-off values of SUVmax and TLuG were selected with the maximum value of Youden index (Youden index=sensitivity+specificity-1). Subsequently, we rounded these values to a clinically useful value.

RESULTS

Study population

Characteristics of all patients are presented in Table 1. ^{18}F-FDG PET/CT and lung function at baseline and after six months of infliximab treatment were available from all 27 patients with pulmonary refractory sarcoidosis, with the exception of DLCOc in 3 patients.

Table 1. Characteristics of patients, n=27

		Patient characteristics
Age		48.1±10.0 years
Gender (male)		17 (63.0%)
Caucasian		24 (88.9%)
Smoking history	Current	4 (14.8%)
	Former	13 (48.1%)
	None smoker	10 (37.0%)
Scadding stages at initiation of infliximab	I	1 (3.7%)[a]
	II	5 (18.5%)
	III	4 (14.8%)
	IV	17 (63%)

[a] Treatment indication for infliximab in this patient was severe obstructive pulmonary function caused by endobronchial stenosing.

Lung function parameters and both semi quantitative metabolic values on ^{18}F-FDG PET/CT, SUVmax and TLuG, at baseline and after 26 weeks of infliximab therapy, are shown in Table 2. After 6 months of treatment with infliximab FVC and FEV1 significantly increased, +4.6% and +5.1% predicted respectively (p=0.009 and p=0.001). Furthermore, the DLCOc increased with +2.4%, however this did not reach significance.

Table 2. Pulmonary function, SUVmax and TLuG at baseline and after 26 weeks infliximab treatment, n=27; mean±SD

	Baseline	**After 26 weeks infliximab**	**Change**	**P-value**
FVC (% predicted)	75.1±18.4	79.7±19.9	+4.6±8.4	0.009
FEV1 (% predicted)	58.6±17.9	63.6±20.5	+5.1±6.8	0.001
DLCOc (% predicted)[a]	55.5±17.9	57.9±16.9	+2.4±6.8	0.100
SUVmax	8.2±4.7	3.1±2.9	-5.1±5.1	<0.001
TLuG	5395±3216	2641±952	-2755±3064	<0.001

[a:] 3 missing values.
FVC= forced vital capacity, FEV1= forced expiratory volume in 1 second, DLCOc= diffusing capacity of the lung for carbon monoxide corrected for haemoglobin; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis.

Both SUVmax and TLuG in the lung parenchyma reduced significantly after therapy with infliximab. SUVmax decreased with 59% from 8.2 ± 4.7 to 3.1 ± 2.9,(p<0.001). TLuG decreased with 51% from 5395 to 2641(p<0.001). There was a very high interobserver agreement for TLuG measurements and SUVmax measurements, with an intraclass correlation coefficient of 0.963 (CI interval 0.917-0.983) (p<0.001) for TLuG and an intraclass coefficient of 0.956 (CI interval 0.906-0.980) for SUVmax.

Correlation between quantification of inflammatory activity measured by ^{18}F-FDG PET and lung function

Correlations between the change in SUVmax, TLuG and the change in lung function parameters are shown in Table 3.

Table 3. Correlation of the change in SUVmax and TLuG with the change in lung function parameters; n=27

Correlation tested R (p-value)	ΔSUVmax	ΔTLuG
ΔFVC	-0.497 (p=0.008)	-0.430 (p=0.025)
ΔFEV1	-0.467 (p=0.014)	-0.532 (p=0.004)
ΔDLCOc	-0.391 (p=0.059)	-0.423 (p=0.039)

Δ= change before and after infliximab therapy; FVC= forced vital capacity, FEV1= forced expiratory volume in 1 second, DLCOc= diffusing capacity of the lung for carbon monoxide corrected for haemoglobin; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis.

Change in SUVmax and TLuG during infliximab therapy correlated significantly (r= 0.735, p<0.001), Figure 2.

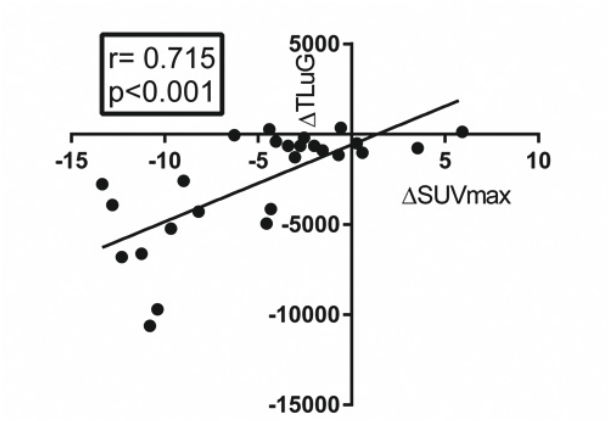


Figure 2. Correlation of the change in SUVmax and TLuG.

Significant correlation was found between ΔSUVmax and ΔFVC during therapy (r= -0.497, p=0.008) and between ΔSUVmax and ΔFEV1 (r=-0.467, p=0.014). No correlation was found between ΔSUVmax and ΔDLCOc. ΔTLuG and ΔFVC as well as ΔTLuG and ΔDLCOc showed a correlation (r= -0.430, p=0.025, and r=-0.423, p=0.039 respectively). In addition, a significant correlation was also found between ΔTLuG and ΔFEV1 (r= -0.532, p=0.004).

Prognostic value of baseline TLuG and SUVmax

Correlations between the baseline SUVmax and TLuG and the change of lung function parameters are shown in Table 4.

Table 4. Correlation of baseline SUVmax and TLuG with change in lung function parameters; n=27

Correlation tested R (p-value)	Baseline SUVmax	Baseline TLuG
ΔFVC	0.460 (p=0.016)	0.323 (p=0.100)
ΔFEV1	0.344 (p=0.079)	0.430 (p=0.025)
ΔDLCOc[a]	0.513 (p=0.010)	0.453 (p=0.026)

[a] 3 missing values

Δ= change before and after infliximab therapy; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis; FVC= forced vital capacity, FEV1= forced expiratory volume in 1 second, DLCOc= diffusing capacity of the lung for carbon monoxide corrected for haemoglobin.

Significant correlation was found between baseline SUVmax and ΔFVC (r=0.460, p=0.016) as well as baseline SUVmax and ΔDLCOc (r=0.513, p=0.010). No correlation was found between baseline SUVmax and ΔFEV1. No correlation was found between baseline TLuG and ΔFVC, although a significant correlation was found between baseline TLuG and ΔFEV1 (r=0.430, p=0.025) and baseline TLuG and ΔDLCOc (r=0.453, p=0.026).

ROC-curves were determined in order to select the best cut-off value of SUVmax and TLuG to predict lung functional response of 5% predicted FVC, FEV1 and DLCOc. The optimal cut-off value of SUVmax to predict a 5% response in FVC, was 7.5, with an AUC of 0.773 (95% CI 0.594-0.951), p=0.018. And the optimal cut-off value of SUVmax to predict 5% response in DLCOc was 9.2 with an AUC of 0.763 (95% CI 0.557-0.698) respectively, p=0.034. The optimal cut-off value of TLuG to predict response of 5% FVC and FEV1 was 4100, with an AUC of 0.739 (95% CI 0.540-0.937) and 0.739 (95% CI 0.544-0.934), p=0.038 and p=0.035. Furthermore, the optimal cut-off value of TLuG to predict response of 5% DLCOc was 4500, with an AUC of 0.744 (95% CI 0.542-0.947), p=0 .049.

Discordant response

A discordant response was shown in only 4 patients, Table 5. One patient showed a decrease in SUVmax, whereas the TLuG increased. And in three patients the SUVmax increased, whereas a decrease in TLuG was shown. Figure 3 shows an example of a patient with a discordant response.

Table 5. Discordant response in SUVmax and TLuG in four patients

	SUVmax			TLuG			ΔLung function		
	pre	**post**	**Δ**	**pre**	**post**	**Δ**	**ΔFVC**	**ΔFEV1**	**ΔDLCoc**
Pt A	11.2	14.7	+32.4%	6606	5826	-11.8%	-0.7%	-5.1%	+9.8%
Pt C	2.1	1.6	-23.4%	1827	2178	+19.2%	-4.1%	-5.3%	0.0
Pt D	6.2	6.8	+9.7%	4616	3596	-22.1%	-10.7%	-8.1%	-4.8%
Pt E	0.6	0.9	+50%	2999	2555	-16.8%	-16.7%	-2.2%	+4.1%

Δ= change before and after infliximab therapy; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis; FVC= forced vital capacity, FEV1= forced expiratory volume in 1 second, DLCOc= diffusing capacity of the lung for carbon monoxide corrected for haemoglobin.

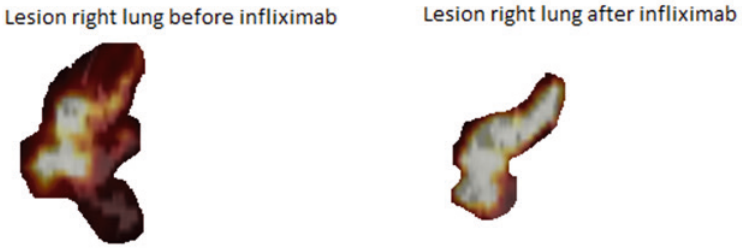


Figure 3. Patient A. Pre- and post-18F-FDG PET/CT, lesion in the right lung with a significant decrease in TLuG and a significant increase in DLCOc (% predicted), while SUVmax increases. Patient A showed persistent extensive parenchymal and endobronchial involvement with an impaired diffusing capacity despite corticosteroid treatment. Infliximab was initiated, and after 6 months of treatment, diffusing capacity increased with 9.8%. Furthermore, TLuG decreased with 11.8%. However, in contrast to TLuG, the SUVmax increased with + 32.4%.

DISCUSSION

In recent years, multiple studies in sarcoidosis patients have described the use of SUVmax to quantify the sarcoidosis activity on a ^{18}F-FDG PET as a biomarker[16,17]. TLuG was determined by two observers and showed a very high interobserver agreement, which implicates that TLuG measurements are reliable. This study demonstrates that change in SUVmax and change in TLuG correlate with change in lung function. No significant difference in correlation coefficient was found. This indicates that both

semi-quantitative values, SUVmax and TLuG, can be used to monitor respiratory response to third line treatment in sarcoidosis with infliximab. A discordant response in TLuG and SUVmax was seen in only 4 of the 27 patients, whereas we hypothesised that TLuG would be a more sensitive marker for response measuring than SUVmax. The discordant response in those 4 patients could be due to different patterns of parenchymal involvement in pulmonary sarcoidosis. For example, in patients with diffuse alveolar sarcoidosis TLuG might be a better parameter, whereas in patients with one or more dense parenchymal infiltrates with high metabolic activity, SUVmax could be a better reflection of disease activity. Moreover, a discordant response can be found in patients with extensive involvement of the lung parenchyma. When the extent of the lesion decreases after therapy, the TLuG decreases while the maximum intensity of ^{18}F-FDG uptake in one pixel, i.e. SUVmax, may remain unchanged.

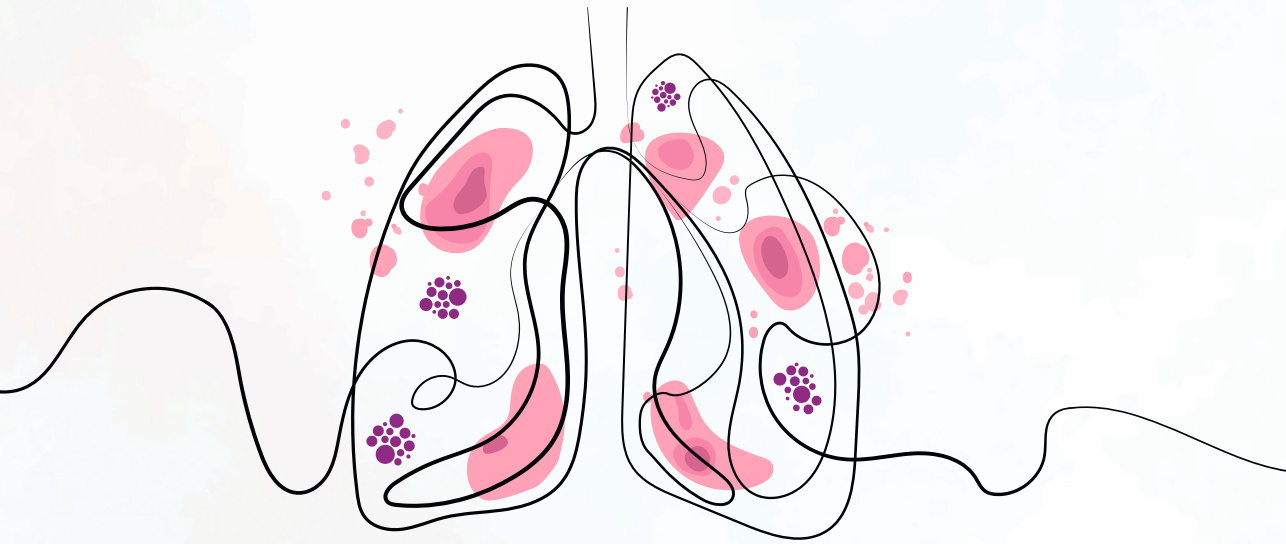
In the second part of our study we evaluated the prognostic value of SUVmax and TLuG at baseline to predict the change in lung function during infliximab treatment. Earlier studies have already focused on the prognostic value of ^{18}F-FDG PET/CT in sarcoidosis. Adams et al. showed that SUVmax is a predictor for future deterioration of the diffusing capacity of the lung[18]. This study has a few limitations. First, the small sample size of the cohort reduces the power of the study. Also, due to a retrospective design of this study there were a few missing data. In addition, long-term follow-up data is only available from a part of the patients in the study cohort, therefore it remains unknown if TLuG is predictive for disease relapse, as previously shown for SUVmax.

CONCLUSIONS

In conclusion, SUVmax and TLuG are both adequate markers to quantify the metabolic response to infliximab in pulmonary sarcoidosis patients. Both SUVmax and TLuG correlate with lung function change during therapy. In addition, SUVmax and TLuG can predict lung functional improvement to be achieved by infliximab. In contrast with our hypothesis TLuG was not superior compared to SUVmax. Based on these data, we recommend to use SUVmax over TLuG in evaluating sarcoidosis activity in the lung parenchyma.

REFERENCES

1. Iannuzzi, M. C. & Fontana, J. R. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* **305**, 391–399 (2011).
2. De Smet, D. *et al.* Use of likelihood ratios improves interpretation of laboratory testing for pulmonary sarcoidosis. *Am. J. Clin. Pathol.* **134**, 939–947 (2010).
3. Grutters, J. C. *et al.* Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest* **124**, 186–195 (2003).
4. Popevic, S. *et al.* Verifying Sarcoidosis Activity: Chitotriosidase versus ACE in Sarcoidosis - a Case-control Study. *J. Med. Biochem.* **35**, 390–400 (2016).
5. Keijsers, R. G. *et al.* 18F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis. *Eur. J. Nucl. Med. Mol. Imaging* **36**, 1131–1137 (2009).
6. Mostard, R. L. M. *et al.* Inflammatory activity assessment by F18 FDG-PET/CT in persistent symptomatic sarcoidosis. *Respir. Med.* **105**, 1917–1924 (2011).
7. Teirstein, A. S. *et al.* Results of 188 whole-body fluorodeoxyglucose positron emission tomography scans in 137 patients with sarcoidosis. *Chest* **132**, 1949–1953 (2007).
8. Nishiyama, Y. *et al.* Comparative evaluation of 18F-FDG PET and 67Ga scintigraphy in patients with sarcoidosis. *J. Nucl. Med.* **47**, 1571–1576 (2006).
9. Wahl, R. L., Jacene, H., Kasamon, Y. & Lodge, M. A. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. *J. Nucl. Med.* **50 Suppl 1**, (2009).
10. Liao, S. *et al.* Prognostic value of metabolic tumor burden on 18F-FDG PET in nonsurgical patients with non-small cell lung cancer. *Eur. J. Nucl. Med. Mol. Imaging* **39**, 27–38 (2012).
11. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **16**, 149–173 (1999).
12. Boellaard, R. *et al.* FDG PET/CT: EANM procedure guidelines for tumour imaging: version 2.0. *Eur. J. Nucl. Med. Mol. Imaging* **42**, 328–354 (2015).
13. Vorselaars, A. D. *et al.* Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur. Respir. J.* **46**, 175–185 (2015).
14. Adams, H. *et al.* Volumetric FDG PET analysis of global lung inflammation: new tool for precision medicine in pulmonary sarcoidosis? *Sarcoidosis, Vasc. Diffus. Lung Dis.* **35**, 44 (2018).
15. Altman, D. *Practical statistics for medical research.* (1999).
16. Cremers, J. *et al.* Extent of disease activity assessed by 18F-FDG PET/CT in a Dutch population. *Sarcoidosis Vasc Diffus. Lung Dis .* **31**, 37–45 (2014).
17. Sobic-Saranovic, D. *et al.* The utility of 18F-FDG PET/CT for diagnosis and adjustment of therapy in patients with active chronic sarcoidosis. *J. Nucl. Med.* **53**, 1543–1549 (2012).
18. Keijsers, R. *et al.* 18F-FDG PET as a predictor of pulmonary function in sarcoidosis. *Sarcoidosis Vasc Diffus. Lung Dis .* **28**, 123–9 (2011).



CHAPTER 6

EFFICACY AND SAFETY OF INFLIXIMAB BIOSIMILAR INFLECTRA® IN SEVERE SARCOIDOSIS

M.C. Schimmelpennink, A.D.M. Vorselaars, F.T. van Beek, H.A. Crommelin, V.H.M. Deneer, R.G.M. Keijsers, M. Veltkamp

Respir. Med. 2018 May;138S:S7-S13

ABSTRACT

Background

Infliximab, a monoclonal antibody against tumour necrosis factor alpha (TNF-α) is an effective third-line therapy in severe sarcoidosis. The originator product of Infliximab, Remicade®, is expensive, limiting universal access. Recently, a less expensive biosimilar of infliximab, Inflectra®, has become available, but the efficacy and tolerability has not been studied in sarcoidosis.

Methods

In this retrospective cohort study, 29 patients treated with the infliximab biosimilar Inflectra®, were analysed**.** Patients received Inflectra® intravenously monthly at a dose of 5mg/kg. We measured trough levels before every infusion. Before and after 6 months of induction therapy pulmonary function and disease activity were evaluated using standardised uptake value (SUV) of the ^{18}F-fluorodeoxyglucose by positron emission tomography (^{18}F-FDG PET), soluble interleukin-2 receptor (sIL-2R), angiotensin converting enzyme (ACE) and health-related quality of life (HRQoL).

Results

In patients with pulmonary sarcoidosis as main treatment indication (n=15) the predicted FVC improved with 8.1%, $p<0.05$. Furthermore, in the whole group HRQoL improved significantly ($p<0.001$), whereas SUVmax and sIL-2R significantly reduced ($p<0.001$ and $p=0.001$, respectively). Hospitalisation due to infections occurred in four patients. None of the patients discontinued Inflectra® due to side-effects. Furthermore, all patients had detectable trough levels indicating development of neutralising antibodies.

Conclusion

Infliximab biosimilar Inflectra® seems effective in the treatment of refractory sarcoidosis with a comparable safety profile to the reference product Remicade®. Inflectra® can be considered as an alternative and less expensive option for patients with refractory sarcoidosis.

BACKGROUND

Sarcoidosis is a multisystem granulomatous disease of unknown aetiology. Moreover, the course and prognosis of sarcoidosis is heterogeneous. Not all patients with sarcoidosis require therapy: most patients have a spontaneously resolving course of disease, while in others the disease can be progressive and even life-threatening[1]. Treatment of sarcoidosis has a multistep approach. Corticosteroids have proved to be effective as initial treatment for sarcoidosis[2–4]. In steroid-refractory cases or in the presence of steroid-associated side effects second-line treatment can be commenced using drugs such as methotrexate, azathioprine, mycophenolate or leflunomide[5–9].

Nevertheless, in some sarcoidosis patients the available first- and second-line therapeutics do not provide the optimal result. In those refractory sarcoidosis patients third-line therapy with targeted TNF-α (tumour necrosis factor alpha) inhibition can be considered[10].

Several randomised clinical trials[11–14] and retrospective studies[15–18] have shown the efficacy of infliximab in refractory sarcoidosis using the originator product Remicade®.

The expensive therapy with anti-TNF-α agents remains a large issue in health care costs. However, with the expiring of the patent of Remicade®, biosimilars of infliximab have become available. Biosimilars are comparable to its reference product in terms of quality, safety and efficacy[19]. Various reports described promising results of the use of biosimilars of the originator product Infliximab (Remicade®), in the treatment of rheumatoid arthritis, psoriasis, ankylosing spondylitis and inflammatory bowel disease[20–23]. Furthermore, the European Medicines Agency (EMA) considers Inflectra® similar to its reference product in efficacy and safety based on two trials: the PLANETA study in patients with ankylosing spondylitis and the PLANETRA study in patients with Rheumatoid Arthritis[20,22]. However, in these studies a much lower dose of Inflectra® and a higher dose of methotrexate was used than currently used in sarcoidosis. Furthermore, it is uncertain whether these data can be extrapolated to the use of the biosimilar Inflectra® in refractory sarcoidosis.

A third point of concern of introducing biosimilars in sarcoidosis is immunogenicity. Formation of neutralizing drug antibodies is related to low trough infliximab serum levels and subsequently are associated with treatment failure[24].

In this study, we report the first cohort of sarcoidosis patients treated with the infliximab biosimilar Inflectra® addressing efficacy and safety.

METHODS

This study is a retrospective cohort study. In 2015 an update of the position paper how to use TNF-α blockers in sarcoidosis patients was published by the Dutch Association of Pulmonologists. In this paper, it was recommended to treat patients with an indication for TNF-α blockers with Inflectra® instead of Remicade® in order to reduce the health care costs and increase accessibility to this drug[25,26]. Therefore, since November 2015 all patients with refractory sarcoidosis with an indication for third-line therapy were started on the biosimilar Inflectra® in our hospital.

Sarcoidosis was defined as refractory when organ damage persisted while receiving second-line immunosuppressive treatment (table 1). Furthermore, refractory sarcoidosis was also defined when second-line therapy had to be discontinued due to toxicity.

Table 1. Definition of refractory sarcoidosis

Refractory sarcoidosis was defined when despite first and second line treatment the following occurred:	**N (number of patients)**
Progressive pulmonary sarcoidosis defined by a decrease of FVC>5% of predicted and/or decrease DLCOc>5% of predicted	5
Progressive pulmonary fibrosis in the context of persistent inflammatory activity defined by positive PET-scan	10
Persistent inflammatory activity of cardiac localisation defined by positive PET-scan	3
Persistent symptomatic sarcoidosis of central nervous system	8
Persistent severe pain due to small fibre neuropathy or osteolytic lesions	3

FVC= forced vital capacity; DLCOc= diffusing capacity of the lung for carbon monoxide corrected for haemoglobin; PET= positron emission tomography

In this study all patients received an intravenous infusion of 5mg/kg Inflectra® at weeks 0 and 2, and subsequently every four weeks. Sarcoidosis was diagnosed when clinical findings were supported by histologic evidence, and after exclusion of other causes of granuloma[1].

The following data from patients were registered: sex, age at the start of Inflectra®, ethnicity, smoking history, prior and current immunosuppressive drug use, duration of disease, main treatment indication and, extra-pulmonary manifestations. Study data were collected and managed using REDCap electronic data capture tools hosted at St. Antonius Hospital, Nieuwegein[27]. The study was approved by the local institutional review board of St. Antonius Hospital Nieuwegein, the Netherlands, with registration number LTME/Z-12.33.

Organ function

Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCOc) and the six-minute walking distance were determined at baseline and after 26 weeks of Inflectra® treatment as previously described in the study of Vorselaars et al.[18]. An increase of 5% of FVC, FEV1 and DLCOc (% predicted) was considered as clinically relevant.

In patients with extra-pulmonary treatment indications, improvement of organ function (functional response) was based on either:

1. Improvement of neurologic symptoms or improvement of pain;
2. Improvement of lesions in the central nervous system seen on MRI (magnetic resonance imaging);
3. Improvement of cardiac ejection fraction by more than 5%;
4. In patients with positron emission tomography (PET)-positive cardiac localisations and prior arrhythmias, absence of arrhythmias during treatment with Inflectra® was defined as functional response.

Inflammatory activity and health-related quality of life

^{18}F-fluorodeoxyglucose by positron emission tomography/computed tomography (^{18}F-FDG PET/CT), genotype corrected angiotensin converting enzyme (ACE), serum soluble interleukin-2 receptor (sIL-2R) were measured as previously published in the study of Vorselaars et al[18].

A decrease of 40% of the maximum standardised uptake value (SUVmax) and a decrease of 40% of one of the biomarkers was considered as a clinically significant response to Inflectra®[18].

In patients with a pulmonary treatment indication we measured the SUVmax in the lung parenchyma and in the mediastinal/hilar lymph nodes. In patients with cardiac sarcoidosis the SUVmax was measured in the mediastinal (including anterior, visceral and posterior mediastinum) and perihilar region. In patients with neurosarcoidosis, small-fibre neuropathy and skeletal sarcoidosis the SUVmax was measured in target lesions and in mediastinal and hilar lymph nodes.

The health-related quality of life (HRQoL) was determined by the 36-Item Short-Form Health Survey (SF-36) and the Visual Analogue Scale (VAS).

The SF-36 contains eight domains: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health. We used only the subscale physical functioning (as reported by Vorselaars et al.[18]), the scale consists of 10 items and is transformed to a 0 (worse score) to 100 scale (best score)[28]. The dimension physical functioning gives an indication of the limitations of patients due to their physical health. Although in some studies an increase of 5 points of the SF-36 was considered clinically relevant[29], we decided to use a more robust increase of 10 points as clinically relevant as previously published[18].

The VAS is a scale measuring the burden of disease also used in the DAS28 in rheumatology[30], ranging from 0 (worst) to 100 (best)[31]. An increase of 10 points of VAS[32] was also considered as a clinically significant response of HRQoL to Inflectra®.

Adverse events and immunogenicity

Side effects and symptoms were evaluated at every visit to the outpatient clinic. Serious infections side effects were defined as infections associated with death, hospitalisation, or the use of intravenous antibiotics.

Trough infliximab serum levels were measured prior to Inflectra® infusion using enzyme-linked immunosorbent assay (ELISA). Antibodies to infliximab (ATIs) were measured in case of suspected treatment failure or adverse reactions. ATIs were detected using radioimmunoassay as previously described[18].

Composite overall response

Composite overall score was measured as previously published by Vorselaars et al.[18] and includes the following dimensions: functional response, inflammatory response and response of HRQoL. Measurement of these responses was performed as described in the previous paragraphs.

Composite overall response was considered excellent when patients showed improvement in all three dimensions, good when patients showed improvement in two out of three dimensions and response in one dimension was defined as a partial response.

Statistics

A power analysis was performed based on the study of Vorselaars et al.[18], using the following criteria: a response of +6.6%±9.2 of % predicted of FVC, two-sided α level of 0.05, and a power of 80%.

To detect the same improvement in FVC published by Vorselaars et al.[18], we needed 15 patients to be included in the pulmonary group.

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as a proportion with percentage. To evaluate differences between baseline and after 26 weeks of follow-up we used the paired Student's t-test. We performed all our analyses with IBM SPSS Statistics 24.

RESULTS

A total of 35 patients were started on Inflectra® since November 2015 in our hospital, of which 5 patients are still in the six months induction phase and are therefore not evaluated in the final results, figure 1. Also, one patient discontinued Inflectra® during induction phase due to development of a penile carcinoma within 4 weeks after starting Inflectra®. Measurements at baseline of the other 29 patients are shown in table 2.

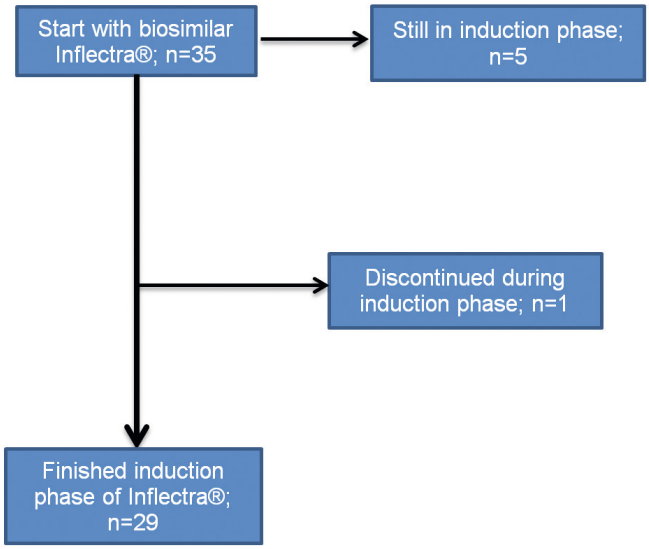


Figure 1. Flowchart of the cohort initiating biosimilar Inflectra®.

Table 2. Baseline characteristics of patients who completed induction phase, n=29

Sex (male), n (%)		15 (51.7%)
Ethnicity (Caucasian), n (%)		23 (79.3%)
Age at initiation of Inflectra® (years), mean (±SD)		49.9±13.0
Duration of disease until initiation of Inflectra® (years), mean(±SD)		6.2±5.5
Diagnosis, n (%)	Biopsy	28 (96.6%)
	Bronchoalveolar lavage	1 (3.4%)*
Smoking status, n (%)	Never	2 (6.9%)
	Current	10 (34.5%)
	Former	17 (58.6%)
Scadding stage, n (%)	Stage 0	5 (17.2%)
	Stage I	3 (10.3%)
	Stage II	10 (34.5%)
	Stage III	5 (17.2%)
	Stage IV	6 (20.7%)
Main treatment indication, n(%)	Pulmonary	15 (51.7%)
	Cardiac	3 (10.3%)
	Small fibre neuropathy	2 (6.9%)
	Central nervous system	8 (27.6%)
	Skeletal sarcoidosis	1 (3.4%)
Use of ≥2 drugs prior to Inflectra®		26 (89.6%)
Medication use prior to initiation of Inflectra®, n (%)	Corticosteroids	29 (100%)
	Methotrexate	23 (79.3%)
	Azathioprine	6 (20.7%)
	Hydroxochloroquine	7 (24.1%)
	Infliximab (Remicade®)	4 (13.8%)
	Adalimumab	1 (3.4%)

* BAL lymphocytosis (25%) with increased CD4+/CD8+ ratio (8.0) in combination with a HRCT-scan pathognomonic for sarcoidosis.

All 29 patients received other systemic therapy before the start of Inflectra® (table 2), however they showed no response or were intolerable to these other treatments. Mean duration in this cohort of second-line treatment prior to Inflectra® was 22 months. Thereafter, third-line treatment with Inflectra® was started, and methotrexate (or corticosteroids) were tapered down and continued in a low dose in order to prevent formation of antibodies.

Mean dose of corticosteroids was tapered down from 16.8mg to 4.3mg. Mean dose of methotrexate was tapered down from 10.8mg to 8.2mg. None of the patients had an

increase in dose of other concomitant immunosuppressive treatments while receiving Inflectra®.

In five patients, sarcoidosis was diagnosed less than a year before starting Inflectra®. All five patients had severe neurosarcoidosis with functional loss without response to corticosteroids and/or methotrexate.

Furthermore, four patients used TNF-α inhibitors before induction of Inflectra®. In three patients using Remicade®, it was discontinued for more than 10 months when Inflectra® was inducted. All patients that received Remicade® showed a good initial response to Remicade®, however they experienced a relapse after discontinuation of therapy that warranted renewed initiation of anti-TNF-α therapy[33]. In the patient treated with adalimumab, Inflectra® was started a month after discontinuation of adalimumab based on antibodies toward adalimumab.

Organ functioning

In patients with a decrease in pulmonary function as main treatment indication (n=15) the FVC significantly improved with +8.1±12.6%, from 74.3±17.6% at baseline to 82.4±24.1% after 26 weeks of treatment with Inflectra®, p=0.026 (table 3 and figure 2a and 2b). Improvement of at least 5% predicted FVC, FEV1 and DLCOc was shown by 8 patients (53%), 8 patients (53%) and 5 patients (33%), respectively.

Table 3. Change in pulmonary function, disease activity, and health-related quality of life after 26 weeks of Inflectra®; Mean (± standard deviation)

	Baseline	After 26 weeks Inflectra®	p-value	Change from the baseline after Inflectra® treatment
Pulmonary function parameters		**Pulmonary group (n=15)**		
Forced vital capacity, % predicted	74.3±18.8	82.4±24.1	<0.05	8.1±12.6
Forced expiratory volume in one second, % predicted	57.3±18.6	64.8±21.3	<0.05	7.5±11.2
Diffusing capacity for carbon monoxide, % predicted	60.9±13.4	66.0±12.2	0.061	5.1±8.1
Six-minute walking distance, % predicted	75.9±19.1	81.3±19.9	<0.05	5.3±9.0
Disease activity and severity measurements		**All patients (n=29)**		
Maximum standardised uptake value mediastinum/ hilar	7.3±4.0	3.7±2.2	<0.001	-3.6±4.8
Maximum standardised uptake value pulmonary parenchyma	6.4±5.2	2.3±1.6	<0.001	-4.1±4.6
Maximum standardised uptake value total	9.3±4.5	3.8±2.5	<0.001	-5.5±5.4
Angiotensin converting enzym (Z-score)	2.19±3.80	0.76±2.08	0.054	-1.43±-0.74
Soluble Interleukin 2-receptor (pg/mL)	7482±9555	4505±8951	0.001	-3319±4413
Health-Related Quality of Life				
Study 36-Item Short-Form Health Survey subscale Physical Functioning	35.3±21.6	45.5±28.1	<0.001	10.3±13.7
Visual analogue scale	34.4±18.6	48.8±20.5	<0.05	14.4±24.0

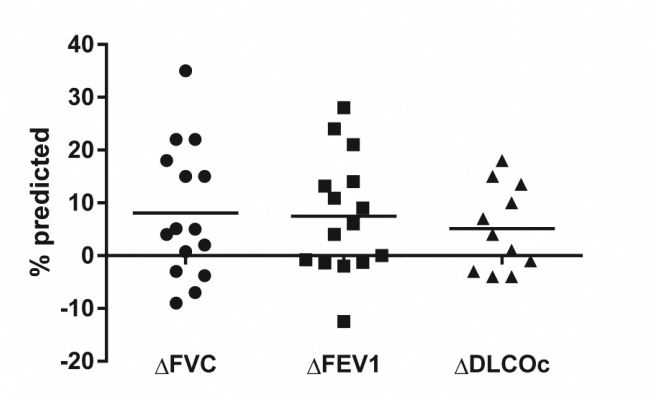


Figure 2a. Change in pulmonary function after 26 weeks of Inflectra® therapy in patients with a pulmonary treatment indication (change in % predicted).
ΔFVC= change in % predicted forced vital capacity; ΔFEV1= change in % predicted forced expiratory volume in one second; ΔDLCOc= change % predicted in diffusing capacity corrected for haemoglobin.

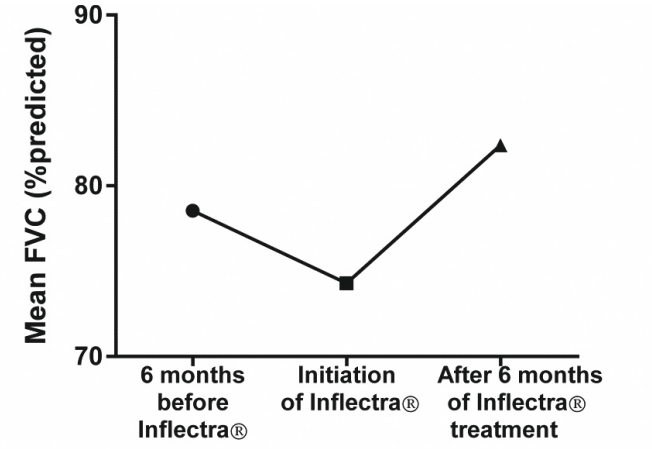


Figure 2b. Mean forced vital capacity (FVC) six months before initiation, at initiation of Inflectra® and after 26 weeks of Inflectra® treatment in patients with a pulmonary treatment indication.

In addition, there was an improvement of the FEV1 (+7.5% predicted) and the six minute walking distance (+5.3% predicted), p= 0.021 and p=0.037, respectively. There was a trend toward improved DLCOc after Inflectra® (+5.3% predicted), p=0.061.

Furthermore, a functional response was seen in 50% of the patients with an extra-pulmonary treatment indication. All three patients with cardiac sarcoidosis as main treatment indication showed a functional response. In three out of 8 patients with

neurosarcoidosis a functional response was seen. Moreover, our cohort included two patients with small fibre neuropathy and one patient with skeletal sarcoidosis, and no functional response was demonstrated in these three patients.

Inflammatory activity

^{18}F-FDG PET/CT at baseline and follow-up after 26 weeks of Inflectra® were available in 28 patients. Six patients showed minimal or no activity on ^{18}F-FDG PET/CT scan at the baseline, of whom two had small fibre neuropathy, three patients had severe neurosarcoidosis and one patient cardiac sarcoidosis.

There was a mean decrease of the SUVmax total of -5.5±5.4, p<0.001 (figure 3A and B). A decrease of at least 40% of baseline of SUVmax was seen in 66% of our patients. Furthermore, a total of 8 patients (28%) even showed a total resolution of inflammatory activity on the PET-scan after the induction phase (Figure 3b). The response rate in the dimension inflammatory activity was 69%.

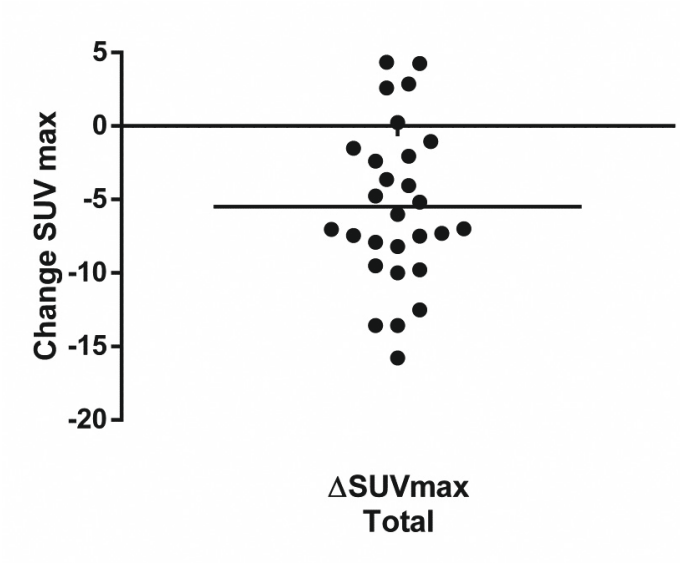


Figure 3a. Change in maximum standardised uptake value after 26 weeks of Inflectra® treatment.

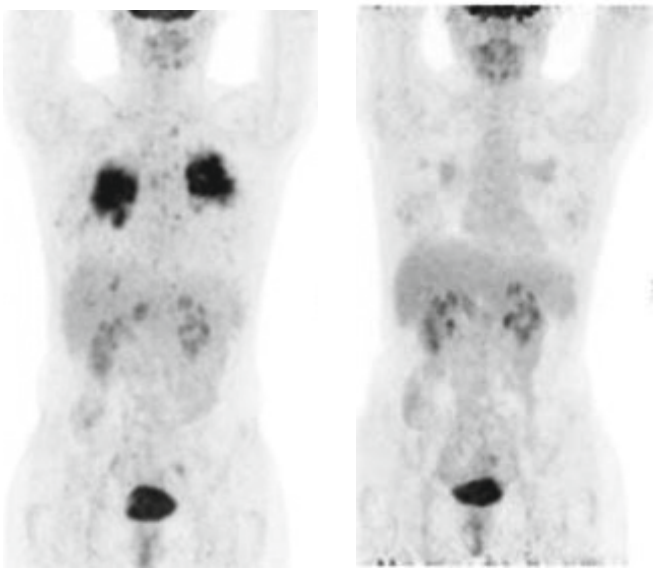


Figure 3b. Example of ^{18}F-FDG PET in a patient with pulmonary sarcoidosis before (left) and after (right) 26 weeks of Inflectra® treatment.

A total of 23 patients showed increased levels of sIL-2R at baseline (figure 4). Biomarker sIL-2R significantly reduced (-3319pg/mL) after treatment with Inflectra®, p<0.001. The z-score of ACE was measured according to the method of Kruit et al.[34]. The z-score of ACE significantly reduced, p=0.030.

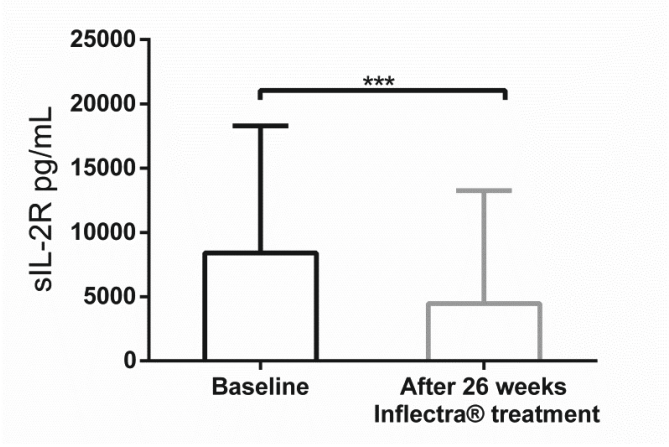


Figure 4. Mean soluble interleukin-2 receptor (sIL-2R) (pg/mL) at the baseline and after 26 weeks of Inflectra® treatment.
*** P≤ 0.001.

Health-related quality of life

Twenty patients finished the SF-36 at baseline and follow-up, 17 patients finished the VAS. After treatment with Inflectra® the mean of SF-36 increased from 35.3 to 45.5, p<0.001 (figure 5). Furthermore, the mean of the VAS significantly improved from 34.4 to 48.8, p=0.025, (figure 5).

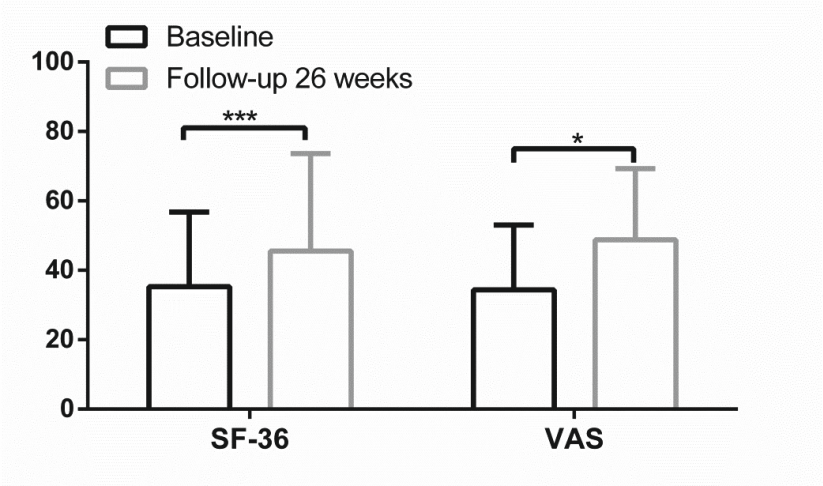


Figure 5. Health-related quality of life at the baseline and after 26 weeks of treatment with Inflectra®: Study 36-Item Short-Form Health Survey (SF-36) subscale Physical Functioning, Visual Analogue Scale (VAS). * P ≤ 0.05; *** P ≤ 0.001.

The composite score describes the following dimensions: functional response, inflammatory response and response of HRQoL, figure 6.

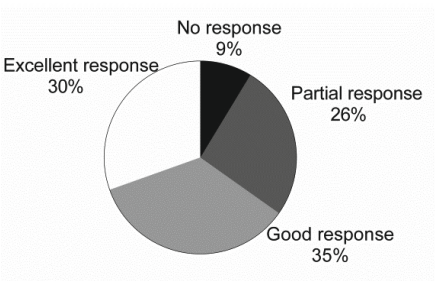


Figure 6a. Response after 26 weeks of Inflectra® therapy .

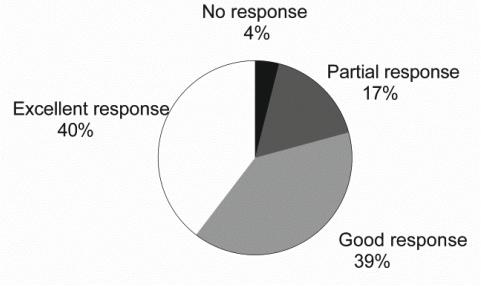


Figure 6b. Response after 26 weeks of Remicade® therapy.

Adverse events and trough levels

Three patients experienced serious side effects based on hospitalisation due to respiratory infections. Furthermore, one patient was hospitalised because of panaritium. All four patients continued with Inflectra® after recovery of the infection. Other side effects were mostly mild infections without hospitalisation. Furthermore, none of the patients experienced leukopenia during therapy with Inflectra®.

The mean trough level in our cohort was 28.3±16.8mg/L. In comparison, in the previously described cohort that was treated with Remicade® in our hospital the mean trough level was 18.4±9.1 mg/L when ATI positive patients were excluded, p=0.011 (figure 7).

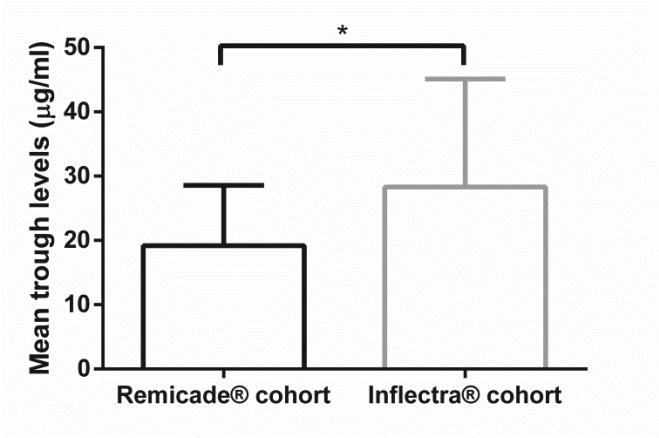


Figure 7. Mean trough levels (mg/L) in the Remicade® cohort versus the Inflectra® cohort. * P ≤ 0.05.

All patients had detectable trough levels, which indicates that there was no formation of antibodies towards Inflectra®.

Methotrexate was given as concomitant immunosuppressant to reduce the risk of ATI formation in 22 patients in a mean dose of 10.5mg/week at initiation of therapy. Two patients used azathioprine in doses of 100mg and 50mg daily, respectively. Furthermore, 15 patients used prednisolone in a mean dose of 16.8/day.

When excluding patients with concomitant immunosuppressant other than methotrexate, the difference between the Remicade® and Inflectra® cohort in trough infliximab serum levels remains significant (data not shown).

DISCUSSION

This is the first report to describe the efficacy and safety of the biosimilar Inflectra® in patients with refractory sarcoidosis. Our data show the positive treatment effect of Inflectra® with an improvement of the pulmonary function and HRQoL. Furthermore, a decrease of disease activity reflected by ^{18}F-FDG PET/CT-scan and biomarkers after treatment with Inflectra® was also demonstrated. In addition, the tolerability for Inflectra® and the safety profile was comparable to the cohort described by Vorselaars et al[18].

When evaluating response after 26 weeks of treatment as a composite of 3 dimensions (functional, inflammatory and quality of life) we found a response rate of 91% in at least one dimension, which seems comparable to the response rate in the Remicade® cohort as previously published. However, when dividing response into three dimensions there were some differences. In the Inflectra® cohort a good response, defined by improvement in 2 or 3 dimensions was seen in 65% which is less compared to the 79% patients with a good response treated with Remicade®.

When comparing both cohorts it is important to state that in the Inflectra® cohort 34.5% of patients had either small fibre neuropathy or neurosarcoidosis and in the Remicade® cohort this was only 19.7%[18]. Neurosarcoidosis and small fibre neuropathy are both difficult to treat which could partly explain the observed differences in the composite scores.

The improvement of pulmonary function in our Inflectra® cohort was comparable with the improvement found in the cohort treated with Remicade®[18]. In concordance with the results of Vorselaars et al.[18], our improvement in FVC also outweighs the improvement of 2.5% FVC found by Baughmann and colleagues[12]. An explanation for this difference could be that in this trial only patients with stable pulmonary sarcoidosis were treated with infliximab. Furthermore, we selected our patients using the ^{18}F-FDG PET/CT scan. The ^{18}F-FDG PET/CT scan is a reliable marker of disease activity and has proved to be more sensitive than ACE and sIL-2R[35-37].

When addressing inflammatory disease activity in sarcoidosis using ^{18}F-FDG PET/CT scan and biomarkers our results were also comparable to the results described in the Remicade® cohort[18].

The patients who showed increased activity on ^{18}F-FDG PET/CT scan after therapy with Inflectra® were patients with neurosarcoidosis (n=3), small-fibre neuropathy

(n=1) and cardiac sarcoidosis (n=1). It is known from literature that some patients with neurosarcoidosis are refractory to immunosuppressive therapy[38]. Another explanation could be that there was no inflammatory activity measured on the ^{18}F-FDG PET/CT scan at the baseline in four of these five patients.

In both the Remicade® cohort as well as the current Inflectra® cohort 14% of patients experienced infections. None of the patients in our cohort discontinued Inflectra® due to severe side effects. However, in comparison, in the Remicade® cohort the infections resulted in discontinuation of therapy[18]. Based on this observation, the tolerability and safety profile of the Inflectra® cohort seems not more severe than the safety profile of the Remicade® cohort.

However, due to the small size of our study population, we should interpret these data with caution. In the PLANETRA study, serious adverse events were reported in 13.9% of patients treated with the biosimilar of infliximab and in 15.7% of the patients treated with the originator infliximab, of whom most patients discontinued therapy due to serious adverse events[39]. The patient who discontinued Inflectra® during the induction phase was diagnosed with penile carcinoma after two infusions of Inflectra®. Therefore, we do not think there is causal relationship between the use of Inflectra® and the development of his malignancy. In previous papers about biosimilars there have been some concerns about possible differences in immunogenicity[40,41]. In our cohort all patients had detectable trough infliximab serum levels indicating that there was no clinically relevant formation of neutralizing antibodies. Furthermore, the mean trough level in our cohort was even higher than the mean trough level of the Remicade® cohort[18]. No significant differences were found between the Remicade® cohort and the Inflectra® cohort in body weight of the patients, dose of infliximab, male:female ratio, concomitant drug use and serum albumin which could explain the difference.

Recently, the NOR-SWITCH trial showed no significant differences in efficacy and safety between the patients who continued treatment with the originator product infliximab Remicade® and patients who switched to the biosimilar Inflectra® in patients with inflammatory bowel disease, spondyloarthritis, rheumatoid arthritis, psoriatic arthritis and chronic plaque psoriasis[42]. These results implicate that it is safe to switch patients from the originator product Remicade® to the biosimilar Inflectra® in indications approved by the EMA. Sarcoidosis is, according to the EMA, not an official treatment indication for Inflectra®. Future prospects in this field are to explore the option of switching of the originator infliximab Remicade® to the biosimilar Inflectra® in patients with refractory sarcoidosis. Taken into account the data from the NOR-switch study

and our data it might be safe to switch from the originator product to the biosimilar in sarcoidosis patients currently treated with Remicade®.

This study has a few limitations. First of all, this study has a small number of patients. Therefore, this study cannot be seen as a non-inferiority trial. However, we performed a power analysis to detect the same difference in FVC as in the Remicade® cohort[18]. Additionally, due to the fact that severe sarcoidosis is a rare condition, a non-inferiority trial is not feasible. Secondly, this study is a retrospective study, and this study design has the possibility of recall bias. However, this is the only available evidence on the use of Inflectra® in sarcoidosis thus far.

In conclusion, the response rate and safety profile of Inflectra® seems comparable to that of Remicade®. Inflectra® may be an alternative and less expensive option for patients with refractory sarcoidosis. Future research should focus on switching to Inflectra® in sarcoidosis patients who are currently being treated with Remicade®.

REFERENCES

1. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **16**, 149–173 (1999).
2. Israel, H. L., Fouts, D. W. & Beggs, R. A. A controlled trial of prednisone treatment of sarcoidosis. *Am. Rev. Respir. Dis.* **107**, 609–614 (1973).
3. James, D. G., Carstairs, L. S., Trowell, J. & Sharma, O. P. Treatment of sarcoidosis. Report of a controlled therapeutic trial. *Lancet (London, England)* **2**, 526–528 (1967).
4. Pietinalho, A., Tukiainen, P., Haahtela, T., Persson, T. & Selroos, O. Early treatment of stage II sarcoidosis improves 5-year pulmonary function. *Chest* **121**, 24–31 (2002).
5. Baughman, R. P., Winget, D. B. & Lower, E. E. Methotrexate is steroid sparing in acute sarcoidosis: results of a double blind, randomized trial. *Sarcoidosis, Vasc. Diffus. Lung Dis. Off. J. WASOG* **17**, 60–66 (2000).
6. Baughman, R. P. & Lower, E. E. The effect of corticosteroid or methotrexate therapy on lung lymphocytes and macrophages in sarcoidosis. *Am. Rev. Respir. Dis.* **142**, 1268–1271 (1990).
7. Vorselaars, A. D. M. *et al.* Methotrexate vs azathioprine in second-line therapy of sarcoidosis. *Chest* **144**, 805–812 (2013).
8. Hamzeh, N. *et al.* Efficacy of mycophenolate mofetil in sarcoidosis. *Respir. Med.* **108**, 1663–1669 (2014).
9. Sahoo, D. H. *et al.* Effectiveness and safety of leflunomide for pulmonary and extrapulmonary sarcoidosis. *Eur. Respir. J.* **38**, 1145–1150 (2011).
10. Baughman, R. P. & Grutters, J. C. New treatment strategies for pulmonary sarcoidosis: antimetabolites, biological drugs, and other treatment approaches. *Lancet. Respir. Med.* **3**, 813–822 (2015).
11. Rossman, M. D. *et al.* A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. *Sarcoidosis Vasc. Diffus. Lung Dis.* **23**, 201–208 (2006).
12. Baughman, R. P. *et al.* Infliximab Therapy in Patients with Chronic Sarcoidosis and Pulmonary Involvement. *Am J Respir Crit Care Med* **174**, 795–802 (2006).
13. Baughman, R. P. *et al.* Infliximab for chronic cutaneous sarcoidosis: a subset analysis from a double-blind randomized clinical trial. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **32**, 289–295 (2016).
14. Judson, M. *et al.* Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomised trial. *Eur Respir J.* **31**, 1189–96 (2008).
15. Cohen Aubart, F. *et al.* Long-term outcomes of refractory neurosarcoidosis treated with infliximab. *J. Neurol.* **264**, 891–897 (2017).
16. Jamilloux, Y. *et al.* Efficacy and safety of tumor necrosis factor antagonists in refractory sarcoidosis: A multicenter study of 132 patients. *Semin. Arthritis Rheum.* **47**, 288–294 (2017).

17. Van Rijswijk, H. N. A. J. *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* **1**, 437–443 (2013).

18. Vorselaars, A. D. *et al.* Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur. Respir. J.* **46**, 175–185 (2015).

19. European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on similar biological medicinal products. (2014).

20. Yoo, D. H. *et al.* A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: The PLANETRA study. *Ann. Rheum. Dis.* **72**, 1613–1620 (2013).

21. Dapavo, P., Vujic, I., Fierro, M. T., Quaglino, P. & Sanlorenzo, M. The infliximab biosimilar in the treatment of moderate to severe plaque psoriasis. *J. Am. Acad. Dermatol.* **75**, 736–739 (2016).

22. Park, W. *et al.* A randomised, double-blind, multicentre, parallel-group, prospective study comparing the pharmacokinetics, safety, and efficacy of CT-P13 and innovator infliximab in patients with ankylosing spondylitis: the PLANETAS study. *Ann. Rheum. Dis.* **72**, 1605–1612 (2013).

23. Razanskaite, V. *et al.* Biosimilar Infliximab in Inflammatory Bowel Disease: Outcomes of a Managed Switching Programme. *J. Crohns. Colitis* **11**, 690–696 (2017).

24. Radstake, T. R. D. J. *et al.* Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann. Rheum. Dis.* **68**, 1739–1745 (2009).

25. Drent, M., Grutters, J. & van Hagen, M. Position Paper: behandeling van sarcoïdose met TNF-alfa blokkerend middelen. *Dutch Assoc. Pulmonologists* (2012).

26. Veltkamp, M., Jonkers, R. & Drent, M. Position Paper: Vergelijkbaarheid en plaatsbepaling biosimilars van TNF-alfa blokker Remicade. *Dutch Assoc. Pulmonologists* (2015).

27. Harris, P. A. *et al.* Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J. Biomed. Inform.* **42**, 377–381 (2009).

28. Ware, J., Snow, K., Kosinski, M. & Gandek, B. *SF-36 Health Survey Manual and Interpretation Guide. New England Medical Center The Health Institute.* (1993).

29. Samsa, G. *et al.* Determining clinically important differences in health status measures: a general approach with illustration to the Health Utilities Index Mark II. *Pharmacoeconomics* **15**, 141–155 (1999).

30. Prevoo, M. L. L. *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.* **38**, 44–48 (1995).

31. Williamson, A. & Hoggart, B. Pain: a review of three commonly used pain rating scales. *J. Clin. Nurs.* **14**, 798–804 (2005).

32. Felson, D. T. *et al.* American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum.* **38**, 727–735 (1995).

33. Vorselaars, A. D. *et al.* Prediction of relapse after discontinuation of infliximab therapy in severe sarcoidosis. *Eur. Respir. J.* **43**, 602–609 (2014).

34. Kruit, A. *et al.* ACE I/D-corrected Z-scores to identify normal and elevated ACE activity in sarcoidosis. *Respir. Med.* **101**, 510–515 (2007).

35. Keijsers, R. G. *et al.* 18F-FDG PET, genotype-corrected ACE and sIL-2R in newly diagnosed sarcoidosis. *Eur. J. Nucl. Med. Mol. Imaging* **36**, 1131–1137 (2009).

36. Keijsers, R. *et al.* 18F-FDG PET as a predictor of pulmonary function in sarcoidosis. *Sarcoidosis Vasc Diffus. Lung Dis .* **28**, 123–9 (2011).

37. Keijsers, R. G. M., Verzijlbergen, J. F., Van Diepen, D. M., Van Den Bosch, J. M. M. & Grutters, J. C. 18F-FDG PET in sarcoidosis: an observational study in 12 patients treated with infliximab. *Sarcoidosis Vasc Diffus. Lung Dis.* **25**, 143–9 (2008).

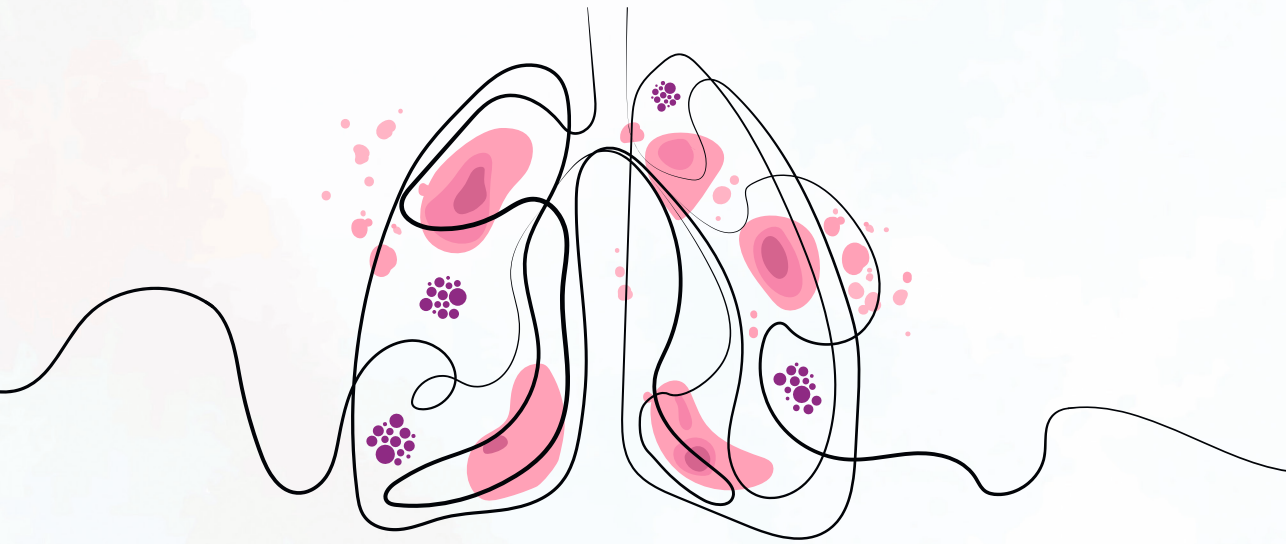
38. Fritz, D., Voortman, M., Van De Beek, D., Drent, M. & Brouwer, M. C. Many faces of neurosarcoidosis: from chronic meningitis to myelopathy. *Curr Opin Pulm Med.* **23**, 439–446 (2017).

39. Yoo, D. H. *et al.* A phase III randomized study to evaluate the efficacy and safety of CT-P13 compared with reference infliximab in patients with active rheumatoid arthritis: 54-week results from the PLANETRA study. *Arthritis Res. Ther.* **18**, (2016).

40. Park, W. *et al.* Comparable long-term efficacy, as assessed by patient-reported outcomes, safety and pharmacokinetics, of CT-P13 and reference infliximab in patients with ankylosing spondylitis: 54-week results from the randomized, parallel-group PLANETAS study. *Arthritis Res. Ther.* **18**, (2016).

41. Faccin, F. *et al.* The design of clinical trials to support the switching and alternation of biosimilars. *Expert Opin. Biol. Ther.* **16**, 1445–1453 (2016).

42. Jørgensen, K. K. *et al.* Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): a 52-week, randomised, double-blind, non-inferiority trial. *Lancet (London, England)* **389**, 2304–2316 (2017).



CHAPTER 7

CHARACTERISATION OF THE PF-ILD PHENOTYPE IN PATIENTS WITH ADVANCED PULMONARY SARCOIDOSIS

M.C. Schimmelpennink, D.B. Meek, A.D.M. Vorselaars, L.C.M. Langezaal, C.H.M. van Moorsel, J.J. van der Vis, M. Veltkamp, J.C. Grutters

Respir Res. 2022 Jun 25;23(1):169

ABSTRACT

Background

Advanced pulmonary sarcoidosis causes significant morbidity and can lead to death. Large trials demonstrated efficacy of antifibrotics in patients with progressive fibrosing interstitial lung diseases (PF-ILD), including a few with sarcoidosis. To date, little is known about this progressive fibrosing phenotype in sarcoidosis. Diffusing capacity of carbon monoxide (DLCO) may be a useful functional marker to screen for advanced pulmonary sarcoidosis. In this study, we describe a cohort with advanced pulmonary sarcoidosis and we gain insights in the progressive fibrosing phenotype in sarcoidosis.

Methods

Patients with sarcoidosis and a DLCO corrected for haemoglobin (DLCOc) <50% predicted were included in this retrospective cohort study. First measurement of DLCOc<50% predicted was the baseline. Lung function data, high-resolution computed tomography (HRCT), pulmonary hypertension (PH) and mortality were collected. Patients with >10% fibrosis on HRCT meeting the criteria for ILD-progression within 24 months were labelled as PF-ILD. With Cox-regression analysis predictors of mortality were established.

Results

106 patients with a DLCOc<50% predicted were included. Evolution of forced vital capacity (FVC) varied widely between patients from -34% to +45% after two years follow-up, whereas change in DLCOc varied between -11% to +26%. Fourteen patients (15%) met the PF-ILD criteria, of whom 6 (43%) died within 10 years versus 10 (13%) in the non PF-ILD group (p=0.006). PH was present 12 (11%), 56 (53%) demonstrated >10% fibrosis on HRCT. Independent predictors of mortality and lung transplantation in the whole cohort are PH, PF-ILD and usual interstitial pneumonia like pattern.

Conclusion

In conclusion, within this group with advanced pulmonary sarcoidosis disease course varied widely from great functional improvement to death. PF-ILD patients had higher mortality rate than the mortality in the overall pulmonary sarcoidosis group. Future research should focus on the addition of antifibrotics in these patients.

INTRODUCTION

Sarcoidosis is a multi-organ granulomatous disorder characterised by a wide variety of clinical phenotypes[1]. About one third of patients with sarcoidosis develop a chronic course of disease[2]. Chronic pulmonary sarcoidosis can cause significant morbidity due to progressive fibrosis, pulmonary hypertension (PH), aspergilloma or other respiratory infections. Strikingly, several studies demonstrated that death rate of sarcoidosis has increased over the last decades[3,4]. Respiratory insufficiency is the main cause of death in sarcoidosis in the western world[4,5]. Advanced age, extensive fibrosis on high-resolution computed tomography (HRCT) and the presence of PH have been identified as predictors of mortality in sarcoidosis[6,7].

Recently, the INBUILD trial revealed that nintedanib slowed disease progression in a heterogeneous group of patients with progressive fibrosing interstitial lung diseases (PF-ILD). The majority of patients consisted of patients with fibrotic hypersensitivity pneumonitis (fHP) and connective tissue disease (CTD)-ILD. This large randomised controlled trial also included several patients with sarcoidosis[8]. The outcomes of the INBUILD trial therefore suggest that treatment with antifibrotic therapy might potentially be useful in patients with sarcoidosis who meet the criteria for PF-ILD. However, nintedanib can cause significant side effects and is relatively expensive and therefore patients to be treated are ideally carefully selected. To date, it is unknown how many patients with sarcoidosis actually meet the criteria for PF-ILD.

The different clinical phenotypes in sarcoidosis make it difficult to predict its course. Walsh and colleagues developed a clinicoradiological risk-stratification system to identify patients with sarcoidosis at risk. This composite score included the following variables: CPI (composite physiological index), main pulmonary artery diameter to ascending aorta diameter ratio (MPAD/AAD ratio) and presence of more than 20% fibrosis on the HRCT[9]. As the authors mention in their paper, the prognostic strength of the CPI might be the incorporation of diffusing capacity (DLCO) to capture increased sensitivity to PH as well as the prognostic effect of DLCO in interstitial lung disease[9]. Given the somewhat laborious formula, the CPI is hardly used in daily practice, whereas the DLCO is an integral part of pulmonary function testing in ILD. The combination of prognostic strength as well as overall availability should make the DLCO a useful tool in screening for advanced sarcoidosis[10]. DLCO negatively correlates with saturation during exercise in patients with ILD[11]. In addition, in patients with a DLCO less than 50% a desaturation of 4% or more was found during exercise[11]. This change in saturation might have a great impact on experiencing dyspnea during exercise. For these reasons, we chose DLCO<50% as entry criteria for selecting patients with advanced pulmonary sarcoidosis.

The aim of this paper is to describe functional and radiological characteristics and prognosis of a cohort with patients with advanced pulmonary sarcoidosis defined by severely limited DLCO. We investigated how many patients with advanced pulmonary sarcoidosis meet the criteria for PF-ILD and the prognosis of this subgroup.

METHODS

In this retrospective study cohort, we included 106 patients with sarcoidosis with at least at one time point a DLCO corrected for haemoglobin (DLCOc) of less than 50% of predicted. All patients with sarcoidosis with a DLCOc<50% of predicted and with an HRCT at the baseline±1year at St. Antonius Hospital Nieuwegein, the Netherlands between 1996 and 2018 were selected. The first time point at which the DLCOc was <50% of predicted in our hospital since the diagnosis sarcoidosis was defined as baseline. Sarcoidosis was diagnosed according to the guidelines of American Thoracic Society/European Respiratory Society/ World Association of Sarcoidosis and other Granulomatous Disorder (ATS/ERS/WASOG)[12].

At the baseline the following data were collected: the lung function, Scadding stage, organ involvement, HRCT data and treatment. Follow-up lung function data were collected one year and two years after the baseline. The following data were also collected: date of diagnosis, date of birth, sex and ethnicity. Research related lab work, e.g. mucin5B *(MUC5B)*, was performed retrospectively from the ILD biobank. Figure 1 pictures the study timeline.

The composite score of Walsh was determined as described in the paper of Walsh et al.[9], and divides the population in patients with a good and poor prognosis. Patients with a CPI higher than 40 were classified as patients with poor prognosis. Patients with CPI ≤ 40, but a MPAD/AAD>1 or extent of fibrosis>20% were also classified as patients with a poor prognosis[9].

The survival period was calculated from baseline to the date of death or lung transplantation, or in the case of survivors to the last known contact. The mortality rate was calculated after 5 and after 10 years. Information concerning vital status of the patient and the cause of death was obtained from patient files or from the general practitioner. Transplant-free survival was defined as survival free of lung-transplantation or death. The study was approved by the Medical research Ethics Committees United (MEC-U) of the St. Antonius Hospital (R05-08A) and all subjects gave written informed consent.

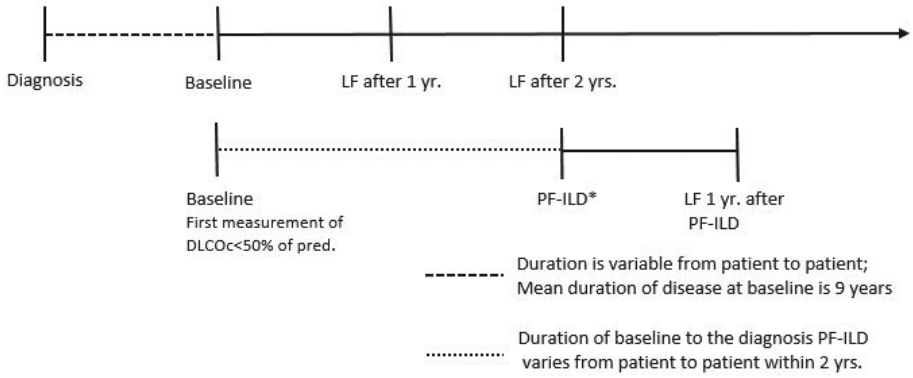


Figure 1. Study timeline.
LF= lung function; DLCOc= diffusing capacity for carbon monoxide corrected for haemoglobin; PF-ILD= progressive fibrosing interstitial lung disease.
*The phenotype PF-ILD was established when the following conditions were met: >10% fibrosis on the HRCT and progressive disease within 24 months of the baseline: a relative decline of FVC of 10% of predicted, a relative decline of FVC of 5-10% of predicted in combination with an increase of fibrosis or worsening of respiratory symptoms, or an increase of fibrosis on HRCT in combination with worsening of respiratory symptoms.

Lung function

The following lung function parameters were collected: forced vital capacity (FVC), forced expiratory volume in one second (FEV1), DLCOc. Finger-prick blood samples were used to estimate venous haemoglobin (Hb) prior to pulmonary function testing. The pulmonary function data were expressed in litres and as percentage of predicted (% pred.). Lung function data were collected from the moment of the first measurement of DLCOc< 50% of predicted (baseline) and after 2 years ± 3 months. In patients with PF-ILD we collected lung function data one year after the diagnosis PF-ILD. CPI at baseline was calculated as previously described by Wells and colleagues[13]: CPI= 91.0 − (0.65 × DLCO % of predicted) − (0.53 × FVC % of predicted) + (0.34 × FEV1 % of predicted).

High-resolution computed tomography

HRCT's were available in all patients at baseline ± 1 year, missing data were handled with pairwise deletion.

A thoracic radiologist with special expertise in ILD (DM) reviewed all HRCT's according to the staging system described by Walsh et al.[9]. In short, the lungs were assessed for the extent of fibrosis, reticulation with or without honeycombing, groundglass and other patterns of disease (not defined as fibrosis or groundglass). The proportion

of the total disease extent and the above mentioned three individual patterns were estimated to the nearest 5%. The radiologist described the absence or presence of traction bronchiectasis and emphysema.

Furthermore, the ratio between the diameter of the mean pulmonary artery and the diameter of the ascending aorta (MPAd/AAd ratio) was measured and subdivided into three categories[9]: 0) diameter of pulmonary trunk less than diameter of ascending aorta; 1) pulmonary trunk diameter/ascending aorta diameter ratio greater than 1 but less than or equal to 1.25; 2) pulmonary trunk diameter/ascending aorta diameter ratio greater than 1.25.

In addition, HRCTs were screened for usual interstitial pneumonia like (UIP-like) pattern as described in the INPULSIS trial[14]. Because the inter-observer agreement for the criteria for UIP is moderate[15], two radiologists screened on a UIP-like pattern. In cases of disagreement a third radiologist made the consensus decision on the presence of UIP-like pattern. A UIP-like pattern was noted if a patient met criteria A and C, B and C or all three criteria A, B and C:

A. Definite honeycomb destruction with basal and peripheral predominance;
B. Presence of reticular abnormality and traction bronchiectasis;
C. Atypical features are absent, specifically nodules and consolidation. Ground glass opacity, if present, is less extensive than reticular pattern.

Mucin 5B

MUC5B promotor polymorphism was analysed as described before in the paper of van der Vis and colleagues[16]. In short, genomic deoxyribonucleic acid was extracted from peripheral blood of each individual using standard method. A pre-designed taqman single nucleotide polymorphism genotyping assay and an ABI 7500Fast analyser (Applied Biosystems, Foster City, CA) were used to genotype rs35705950.

We compared the minor allele frequency of the patients of our cohort with the minor allele frequency of healthy control subjects. In the healthy control group 249 healthy unrelated Dutch Caucasians were included.

Progressive fibrosing interstitial lung disease

PF-ILD was defined as described in the INBUILD trial[8]. All patients with PF-ILD had more than 10% fibrosis on the HRCT. The disease was defined as progressive if the following criteria were met within 24 months of the baseline: a relative decline of FVC of 10% of the predicted value, a relative decline of FVC of 5-10% of the predicted value

in combination with an increase of fibrosis or worsening of respiratory symptoms, or an increase of fibrosis on HRCT in combination with worsening of respiratory symptoms. Patients with incidental decrease of FVC (thus only one measurement of FVC loss followed by an increase of FVC) were not labelled as progressive disease. Our thoracic radiologist (DM) evaluated the HRCT for progression of fibrosis.

We collected the follow-up of lung function data from the moment that a patient met the criteria for PF-ILD until one year thereafter. We have chosen the same follow-up duration of one year as was described in the large INBUILD trial.

Statistics

Statistical analyses were performed using IBM SPSS version 24 and Graphpad prism software version 6.05. Continuous variables are expressed as mean ± standard deviation (parametric data) or median with interquartile range (non-parametric data). Differences between groups with continuous data were tested with Student's test or the Mann-Whitney U test where appropriate. Numeric data are expressed as number (percentage), and differences between non-continuous data were measures with Chi-square test. Survival analysis was performed using the Kaplan-Meier curves. For the composite endpoint overall mortality patients who underwent lung transplantation were considered dead. Univariate Cox regression analysis was used to identify predictors for transplant-free survival. Subsequently, we used multivariate Cox regression analysis in order to demonstrate independent predictors of transplant-free survival. Candidate covariates from the univariate analysis were included for multivariate analysis if $p<0.05$. No missing data substitutions were made. Missing data were handled with pairwise deletion.

RESULTS

The cohort consisted of 106 patients with advanced pulmonary sarcoidosis. The patient characteristics at baseline are outlined in table 1. The mean age of this study cohort was 49 years and 68% of the patients were male. Median duration of disease prior to the first measurement of DLCOc<50% of predicted was 5 years. Mean CPI was 47±9, and a CPI above 40 was measured in 87 patients. According to the algorithm of Walsh 98 patients (92%) are suspected to have a poor prognosis: 87 out of the 98 patients (89%) had a CPI above 40, 8 out of the 98 patients (8%) had more than 20% fibrosis on HRCT and 8 out of the 98 patients (8%) had a MPAd/AAd ratio of more than 1.

Table 1. Patient characteristics at first measurement of DLCOc <50% of predicted

		n=106
Age		49 ± 13 years
Male/Female		72 (68) / 34 (32)
White/ Non-white/Unknown		66 (62) / 30 (28) / 10 (9)
Histologic confirmation		104 (98)
Smoking history	Never	29 (27)
	Former	48 (45)
	Current	23 (22)
	Unknown	6 (6)
Cardiac involvement	Probable	6 (6)
	Possible	2 (2)
≥ 2 organs involved		95 (90)
Therapy	Corticosteroids	59 (56)
	Methotrexate	37 (35)
	Azathioprine	3 (3)
	Plaquenil	4 (4)
	Anti-TNF treatment	8 (8)
	None	26 (25)
	Unknown	4 (4)
Duration of disease prior to baseline time point		5 (13) years
Scadding stage	0	2 (2)
	I	4 (4)
	II	19 (18)
	III	12 (11)
	IV	63 (59)
	Unknown	6 (6)
PH	Diagnosed with RHC	9 (8)
	PH suspected on echocardiogram	3 (3)
FVC % predicted		70 ± 17
FEV1% predicted		60 ± 18
DLCOc% predicted		42 ± 7
CPI		47 ± 9
Walsh poor prognosis		98 (92)
MUC5B promotor polymorphism	GG	83 (78)
	GT	23 (22)
	TT	0

Data are shown as number (%) or mean±SD, except for the duration of disease this is shown as median (IQR).

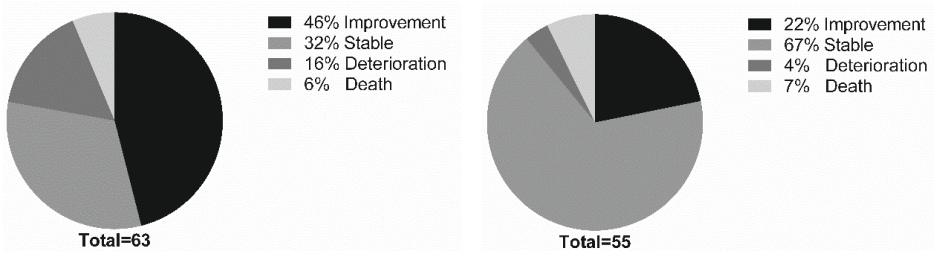
PH= pulmonary hypertension; RHC= right heart catheterization FVC= forced vital capacity; FEV1= forced expiratory volume in one second; DLCOc= diffusing capacity for carbon monoxide corrected for haemoglobin; CPI =composite physiologic index; *MUC5B*= mucin 5B.

In addition, at baseline, 12 patients (11%) were diagnosed with PH based on mPAP (mean pulmonary arterial pressure) >25mmHg during right heart catheterisation (n=9) or compatible findings of transthoracic echocardiography (n=3). The diagnosis of PH using echocardiography was based on a high tricuspid regurgitation maximum velocity of 4.4m/s and secondary signs of pulmonary hypertension in one patient and in two patients based on secondary signs of pulmonary hypertension, such as right atrial dilatation, hypertrophy of right ventricle and paradoxical septal motion. In the patients who received right heart catheterisation mPAP was 38±13mmHg. 76 patients (72%) were treated with immunosuppressive therapy at the baseline. The minor allele frequency for *MUC5B* rs35705950 in our cohort was 11% and did not significantly differ from the minor allele frequency of 9% in the controls (p>0.05).

Change in lung function

Mean FVC at baseline was 70% of predicted and mean DLCOc at baseline was 42% of predicted (table 1). After two years, four patients had died (4%) and FVC and DLCOc were available for 59 and 51 patients, respectively. Evolution of lung function varied widely between patients. Change in FVC after two years follow-up ranged between –34% to +45% of predicted, whereas change in DLCO varied between -11% to +26%.

After 2 years of follow-up 29 patients (46%) had improved FVC and 12 patients (22%) improved DLCOc (figure 2). Deterioration of FVC was in 10 patients (16%), whereas only 2 (4%) had deterioration of DLCOc (figure 2).



A. **B.**

Figure 2. Proportions of patients with improved, stable or deteriorated lung function and patients who died.
A. improvement, stable or deteriorated FVC after two years; B. improvement, stable or deteriorated DLCOc after two years.
FVC: Improvement ≥+5% predicted; Stable -5 to +5% predicted; deterioration ≥-5% predicted.
DLCOc: Improvement ≥+10% predicted; stable -10 to +10% predicted; deterioration ≥-10% predicted.

High-resolution computed tomography

HRCT data at baseline ± 1 year were available in 106 patients (table 2). In this cohort, the median total disease extent of the lung was 70%. The most prominent HRCT pattern was groundglass, with a median of 21% involvement of the lungs. A total of 56 patients had more than 10% fibrosis on HRCT. Furthermore, only four (4%) patients had a UIP-like pattern on HRCT.

Table 2. HRCT characteristics at baseline

HRCT characteristics	n=106
Presence of fibrosis	79 (75)
>10% of the lungs fibrosis	56 (53)
Total disease extent (%)	70; 30-90
Fibrosis; % of lungs	12; 0 – 24
Groundglass; % of lungs	21; 4 – 52
Other pattern; % of lungs	7; 0 – 20
Traction bronchiectasis	59 (56)
Emphysema	22 (21)
MPAd/AAd category: 0/1/2	49 (46)/50 (47)/7 (7)
UIP-like pattern	4 (4)

Data are shown as number (percentage) or median; IQR.
HRCT= high-resolution computed tomography; MPAd/AAd= mean pulmonary artery diameter/ascending aorta diameter; UIP= usual interstitial pneumonia.
MPAd/AAd category 0) MPAd/AAd <1; category 1) MPAd/AAd 1-1.25; category 2) MPAd/AAd >1.25.

Progressive fibrosing interstitial lung disease

In 94 patients we had enough data to apply the criteria for PF-ILD as used in the INBUILD trial. We found that 14 out of the 94 patients (15%) met the PF-ILD criteria.

Lung function data after one year from the moment that patients had PF-ILD showed no significant increase or decrease (figure 3). In two patients follow-up lung function data were not available because they had already died. All patients received immunosuppressive therapy at the time they met the criteria of PF-ILD. Three patients switched immunosuppressive therapy within one year of follow-up.

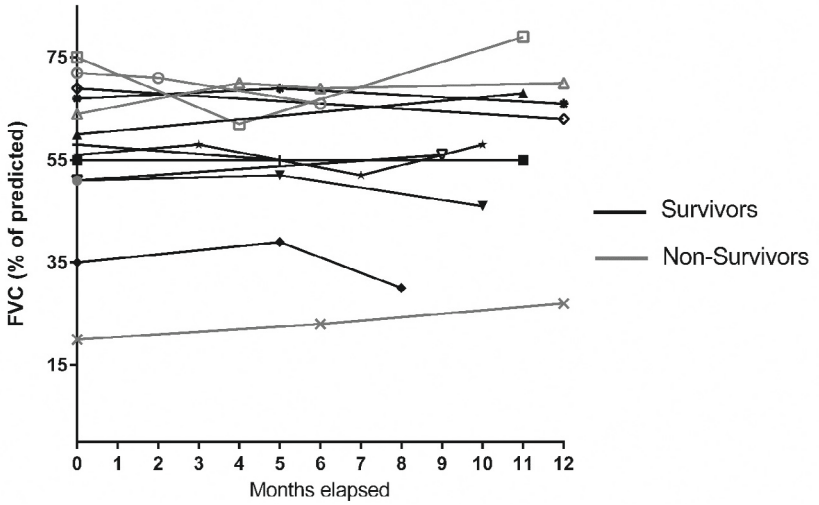


Figure 3. One year follow-up FVC (% of predicted) in patients with PF-ILD.
Time 0 (T=0) is the first time that patients fulfil the criteria for PF-ILD.

No difference was found between survivors and non-survivors in change in FVC after one year follow-up in patients with PF-ILD (p>0.05).

Patients with PF-ILD are characterised by more fibrosis and traction bronchiectasis on HRCT (table 3). Mortality in the PF-ILD group was significantly higher than in the non PF-ILD group (43% versus 13%; p=0.013). One patient underwent a bilateral lung transplantation, 3 patients died because of end-stage fibrotic sarcoidosis and two patients died because of pneumonia in the PF-ILD group. There was no significant difference in *MUC5B* rs35705950 T-allele carriership between PF-ILD and non PF-ILD. However, in the whole cohort (PF-ILD and non PF-ILD) three out of four patients with UIP-like pattern on HRCT carried the *MUC5B* T-allele.

Table 3. Characteristics of patients with PF-ILD versus non PF-ILD at baseline (DLCOc<50% of pred.)

		PF-ILD (n=14)	Non PF-ILD (n=80)	p-value
Age		52±12	49±13	NS
Male		10 (71)	51 (64)	NS
Ethnicity	White	7 (50)	51 (64)	NS
	Non-white	4 (29)	23 (29)	
	Unknown	3 (21)	6 (7)	
Smoking history	Never	5 (36)	20 (25)	NS
	Former	9 (64)	37 (46)	
	Current	0	18 (23)	
	Unknown	0	5 (6)	
CPI		48±14	47±8	NS
PH		4 (29)	8 (10)	NS
Total disease extent		70; 48-90	73; 30-89	NS
% Fibrosis		33; 18-37	8; 0-20	<0.001
% Groundglass		14; 5-25	25; 3-62	NS
% Other pattern		12; 5-18	7; 0-24	NS
MPAd/AAd ratio		1.0; 0.9-1.1	1.0; 0.9-1.1	NS
Emphysema		3 (21)	16 (20)	NS
Traction bronchiectasis		13 (93)	37 (46)	0.001
UIP-like pattern		1 (7)	3 (4)	NS
MUC5B; rs35705950 carriers of T allele (genotype GT or TT)		3 (21)	18 (23)	NS
Deaths/lung transplantation		6 (43)	10 (13)	0.013

Age and CPI are shown as mean ± standard deviation. The rest of the table is shown as number (%) or median; interquartile range.
PF-ILD= progressive fibrosing interstitial lung disease; NS=not significant; CPI=composite physiological index; PH= pulmonary hypertension; MPAd/AAd= mean pulmonary artery diameter/ascending aorta diameter; UIP= usual interstitial pneumonia; *MUC5B*= mucin 5B.

Survival

Univariate analysis

A total of 15 patients died of whom in 14 patients it was definitely attributable to sarcoidosis. In one patient the cause of death was familial dilated cardiomyopathy (not sarcoidosis related). Three patients underwent bilateral lung transplantation, and four patients died on the waiting list.

In the total cohort, the 5- and 10-year overall mortality was 11% and 16%, respectively.

Univariate Cox regression analysis revealed four predictors for the composite endpoint of overall mortality (table 4): CPI (HR 1.1; 95% CI 1.0-1.1; p=0.049), PH (HR 3.8; 95% CI 1.0-14.3; p=0.047), PF-ILD (HR 4.5; 95% CI 1.6-12.5; p=0.004), and a UIP-like pattern on HRCT (HR 9.8; 95% CI 2.6-36.6; p=0.001).

Table 4. Univariate and multivariate analysis of predictors of mortality and lung transplantation

	N	Univariate analysis for overall mortality and lung transplantation			Multivariate analysis for overall mortality and lung transplantation		
		HR	95% CI	p-value	HR	95% CI	p-value
Age	106	1.0	1.0-1.1	0.127			
Gender (male)	106	1.6	0.5-4.8	0.437			
Ethnicity: white	96	0.4	0.1-1.1	0.070			
Smoking Ex	100	1.5	0.5-4.2	0.441			
Current	100	5.0	0.6-38.2	0.124			
CPI	104	1.1	1.0-1.1	**0.049**	1.0	1.0-1.1	0.256
PH	106	3.8	1.0-14.3	**0.047**	4.6	1.1-20.3	**0.042**
PF-ILD	94	4.5	1.6-12.5	**0.004**	4.5	1.5-13.7	**0.008**
Scadding stage IV	100	1.5	0.5-4.0	0.451			
Total disease extension	106	1.0	1.0-1.0	0.438			
% Fibrosis	106	1.0	1.0-1.1	0.062			
% Groundglass	106	1.0	1.0-1.0	0.090			
% Other pattern	106	1.0	1.0-1.0	0.761			
MPAd/AAd ratio	103	3.2	0.2-58.0	0.429			
Emphysema	106	1.0	0.3-3.1	0.987			
Traction bronchiectasis	106	1.3	0.5-3.3	0.627			
UIP-like pattern	106	9.8	2.6-36.6	**0.001**	13.1	3.1-54.6	**<0.001**
MUC5B; carriers of T allele	106	0.6	0.2-2.1	0.442			
Walsh poor prognosis	106	22.6	0.0-190	0.465			

HR= hazard ratio; 95% CI= 95% confidence interval; CPI=composite physiological index; PH= pulmonary hypertension; PF-ILD= progressing fibrosing interstitial lung disease; MPAd/AAd= mean pulmonary artery diameter/ascending aorta diameter; UIP= usual interstitial pneumonia; *MUC5B*= Mucin5B.

Multivariate analysis

The following covariates were analysed with multivariate Cox regression analysis: CPI, PH, PF-ILD and UIP-like pattern. Multivariate Cox regression analysis demonstrated that three covariates are independent predictors of overall mortality and lung transplantation: PH, PF-ILD and UIP-like pattern (table 4). Comparison of transplant-free survival in patients with and without PF-ILD is shown by Kaplan Meier curve in figure 4A and with and without PH in figure 4B.

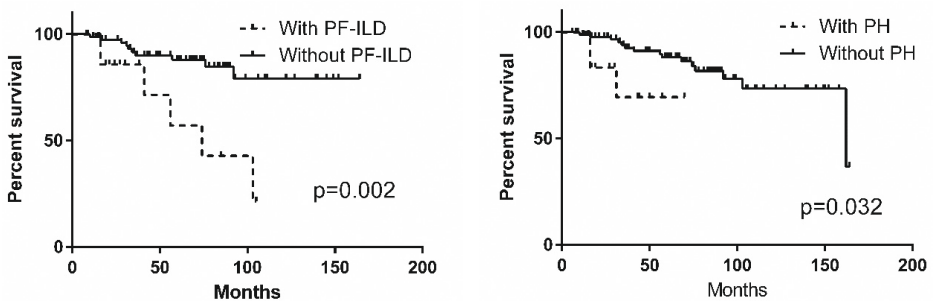


Figure 4. Kaplan Meier curves for the transplant-free survival. A. Survival analysis in patients with and without PF-ILD; B. Survival analysis in patients with and without PH.
Median survival patient with PF-ILD = 6 years; median survival patients with PH = 5 years.

DISCUSSION

In this study in patients with advanced pulmonary sarcoidosis based on a severely impaired DLCOc, we demonstrated that 15% of patients meet the current criteria for PF-ILD. In our study cohort, we found PH, PF-ILD and UIP-like pattern on HRCT to be independent predictors for all-cause mortality and lung transplantation. In the group of sarcoidosis who meet PF-ILD criteria, 43% died or underwent bilateral lung transplantation, whereas the overall mortality in the non-PF-ILD was 13%.

As no well-described definition of advanced pulmonary sarcoidosis exists, we chose to use DLCOc as determining criterion. DLCO is reduced in 90% of the patients with advanced pulmonary sarcoidosis[17]. Furthermore, in the absence of fibrosis DLCO might be limited in patients with sarcoidosis, for example, in patients with PH[18]. An important observation in our study is the significant heterogeneity in disease course. Although a severely impaired DLCO in patients with IPF or CTD-ILD in general is associated with a poor prognosis[19], we see a remarkably high percentage of patients in our study cohort where FVC remains stable (32%) or even improved (46%) after 2 years of follow-up. One explanation could be that patients with sarcoidosis generally respond well to immunosuppressive therapy. In the observational multi-center study of Kampstra et al.[20] including 509 patients with different stages of sarcoidosis was found that 21% of the patients had an improvement of FVC and 17% had an improvement of DLCO after 2 years follow-up. The mean % of DLCOc in our study cohort was 42% which is comparable with mean DLCOc at baseline in, for example, the ASCEND, INPULSIS and INBUILD trial, 44%, 47% and 46% respectively[8,14,21].

We demonstrated that PF-ILD is rare, even in patients with advanced pulmonary sarcoidosis, as our data show that only 15% of our cohort fulfil the criteria of PF-ILD. In the overall sarcoidosis-population, the percentage of patients fulfilling the criteria of PF-ILD would be significantly lower because we used an inclusion criterion of at least one measurement of DLCOc<50% predicted. A retrospective Italian study found that 41% of sarcoidosis patients with Scadding stage IV had PF-ILD, which is much higher than the prevalence what we found. This difference might be explained by the fact that in our study PF-ILD was diagnosed if patients had more than 10% fibrosis on HRCT and met the criteria for progressive disease according to the INBUIDL trial, whereas in the Italian study patients with Scadding stage IV who met the criteria for progressive disease were labelled as PF-ILD[22]. In the patients with PF-ILD we found a mean increase of 105mL FVC after one year follow-up (data not shown), whereas in the placebo-group of the INBUILD trial a mean decrease of -187.8mL after one year was found. However, our data cannot be compared with the outcomes of the INBUILD trial, because in the INBUILD trial tipping point analysis is used to impute FVC data in patients who died. Despite stable FVC after one year follow-up in patients with PF-ILD, our data also demonstrate that PF-ILD in patients with advanced pulmonary sarcoidosis is associated with a poor prognosis given the fact 43% of this group died from sarcoidosis related causes. From these findings, we might conclude that FVC does not give a realistic reflection of the severity of the disease. However, our lung function results should be interpreted with caution as the group composed of only 14 patients. In the study of Behr and colleagues[23], describing the course of disease in IPF patients with and without antifibrotic therapy, was demonstrated that overall decline of FVC did not differ between patients with and without antifibrotic therapy, whereas they found a great significant difference in mortality between patients with and without antifibrotic therapy. CPI (incorporating FVC, FEV1 and DLCO) or six-minute walk test (6MWT) seem more suitable as clinical parameters to evaluate the therapeutic effect in sarcoidosis, as both parameters are clinical predictors of mortality in ILD[9,24]. In the INBUILD trial, patients did not receive other therapy besides nintedanib or placebo, whereas in our cohort all patients with PF-ILD received immune suppressive treatment. Nintedanib is not a curative treatment, and a relatively expensive drug[25] and can have substantial side-effects, and therefore patients and society can benefit from careful selection prior to initiation of this therapy. In the light of the recently published INBUILD trial, we suggest that patients with advanced pulmonary sarcoidosis fulfilling the criteria for PF-ILD might benefit from treatment with nintedanib.

Although the vast majority of the total cohort remained stable or even improved in terms of lung function, 12 patients (11%) eventually died or received lung transplantation within 5 years. Presence of PF-ILD, UIP-like pattern on HRCT and presence of PH

were independent predictors of mortality. Unlike the findings in the United States and France[4,6], we were not able to demonstrate racial or sex differences in mortality. However, this might be because the limited power of this study for mortality. Only a few studies focused on mortality in advanced pulmonary sarcoidosis[5,6]. The overall 5-year mortality including lung transplantation was 11% in our study, which is similar to the results of Nardi and colleagues a 5-year mortality rate of 8.5% and that 4% underwent a lung transplantation[5] in a cohort with patients with only Scadding stage IV. Four out of six patients waiting for lung transplantation died. In a retrospective study, investigating mortality in patients with sarcoidosis on the transplant wait list, 18% died within one year and DLCO was found to be a strong predictor for death[26]. This study in combination with our results underlines the importance of appropriate timing for referral for lung transplantation.

Although 56 patients had more than 10% fibrosis on HRCT, a UIP-like pattern was found in only four patients. A UIP-like pattern is rare in sarcoidosis, therefore the question remains if patients with a UIP pattern in sarcoidosis might have two disorders or that a UIP pattern is a rare consequence of progressive fibrosis in sarcoidosis. The continuous inflammatory condition of the lungs may predispose to the development of a UIP in susceptible patients with sarcoidosis. In addition, pro-fibrotic genetic markers can play a role in the development of a UIP pattern in sarcoidosis. Three out of four patients with a UIP on HRCT carried the *MUC5B* T-allele. In RA-ILD it was shown that UIP is associated with presence of the *MUC5B* T-allele[27]. A similar situation may be present in sarcoidosis although UIP is much less frequent and subsequent studies focusing on this rare phenotype in sarcoidosis are needed to confirm this hypothesis. Thus far, only one other study has investigated the association between *MUC5B* variant and sarcoidosis. Stock et al.[28] demonstrated that *MUC5B* is not associated with fibrotic sarcoidosis (Scadding stage IV) nor with progression of sarcoidosis. However, they did not study the association between the presence of the UIP pattern in fibrotic sarcoidosis and *MUC5B* genotype.

The last independent predictor found for overall mortality was the presence of PH. It is well known that PH is associated with a poor prognosis in sarcoidosis[5–7]. In our cohort with advanced pulmonary sarcoidosis, 11% of the patients had PH at baseline. The incidence of PH in the overall sarcoidosis population varies widely from 3% to 20%[29–33], and in patients awaiting for lung transplantation the incidence is even higher varying from 39% up to 79%[26,34]. In 58% of the patients with PH, fibrosis was found on HRCT. This is in line with the study of Sulica et al.[35], this retrospective study found that 60% of the patients with PH had fibrosis.

As our study is retrospective, lung function and HRCT were not performed at strictly set times, and therefore we had some missing data. However, the aim of our study was to describe a cohort with advanced pulmonary sarcoidosis and we feel that our cohort is a realistic reflection of this phenotype in clinical practice.

CONCLUSION

In conclusion, in this study on advanced pulmonary sarcoidosis defined on the basis of severely lowered DLCOc (<50%), a PF-ILD phenotype was found in 15%. Overall disease course showed remarkable heterogeneity. Although lung function in most patients improved or stabilised, overall 5-year and 10-year mortality in the whole cohort was substantial with 11% and 16%, respectively. Independent negative predictors of transplant-free survival were PH, UIP-like pattern and PF-ILD phenotype. Future research should focus on the efficacy and timing of antifibrotic therapy in patients with sarcoidosis with a PF-ILD phenotype.

REFERENCES

1. Iannuzzi, M. C., Rybicki, B. A. & Teirstein, A. S. Sarcoidosis. *New England Journal of Medicine* vol. 357 2153 (2007).
2. Rubio-Rivas, M. & Corbella, X. Clinical phenotypes and prediction of chronicity in sarcoidosis using cluster analysis in a prospective cohort of 694 patients. *Eur. J. Intern. Med.* **77**, (2020).
3. Swigris, J. J. *et al.* Sarcoidosis-related mortality in the United States from 1988 to 2007. *Am. J. Respir. Crit. Care Med.* **183**, 1524–1530 (2011).
4. Mirsaeidi, M., Machado, R. F., Schraufnagel, D., Sweiss, N. J. & Baughman, R. P. Racial difference in sarcoidosis mortality in the United States. *Chest* **147**, 438–449 (2015).
5. Nardi, A. *et al.* Stage IV sarcoidosis: Comparison of survival with the general population and causes of death. *Eur. Respir. J.* **38**, 1368–1373 (2011).
6. Jeny, F. *et al.* Predictors of mortality in fibrosing pulmonary sarcoidosis. *Respir. Med.* **169**, 105997 (2020).
7. Kirkil, G., Lower, E. E. & Baughman, R. P. Predictors of Mortality in Pulmonary Sarcoidosis. *Chest* **153**, 105–113 (2018).
8. Flaherty, K. R. *et al.* Nintedanib in progressive fibrosing interstitial lung diseases. *N. Engl. J. Med.* **381**, 1718–1727 (2019).
9. Walsh, S. L. F. *et al.* An integrated clinicoradiological staging system for pulmonary sarcoidosis: A case-cohort study. *Lancet Respir. Med.* **2**, 123–130 (2014).
10. Boros, P. W. *et al.* Impaired lung compliance and DL, CO but no restrictive ventilatory defect in sarcoidosis. *Eur. Respir. J.* **36**, 1315–1322 (2010).
11. Kelley, M. A., Panettieri, R. A., Krupinski, A. V & Philadelphia, R. Resting Single-Breath Diffusing Capacity as a Screening Test for Exercise-Induced Hypoxemia. (1986).
12. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **16**, 149–173 (1999).
13. Wells, A. U. *et al.* Idiopathic pulmonary fibrosis: A composite physiologic index derived from disease extent observed by computed tomography. *Am. J. Respir. Crit. Care Med.* **167**, 962–969 (2003).
14. Richeldi, L. *et al.* Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis. *N. Engl. J. Med.* **370**, 2071–2082 (2014).
15. Walsh, S. L. F., Calandriello, L., Sverzellati, N., Wells, A. U. & Hansell, D. M. Interobserver agreement for the ATS/ERS/JRS/ALAT criteria for a UIP pattern on CT. *Thorax* **71**, 45–51 (2016).
16. Van Der Vis, J. J. *et al.* Effect of Muc5b promoter polymorphism on disease predisposition and survival in idiopathic interstitial pneumonias. *Respirology* **21**, 712–717 (2016).
17. Abehsera, M. *et al.* Sarcoidosis with pulmonary fibrosis: CT patterns and correlation with pulmonary function. *Am. J. Roentgenol.* **174**, 1751–1757 (2000).
18. Baughman, R. P. *et al.* Clinical features of sarcoidosis associated pulmonary hypertension: Results of a multi-national registry. *Respir. Med.* **139**, 72–78 (2018).

19. Chan, C., Ryerson, C. J., Dunne, J. V. & Wilcox, P. G. Demographic and clinical predictors of progression and mortality in connective tissue disease-associated interstitial lung disease: A retrospective cohort study. *BMC Pulm. Med.* **19**, (2019).

20. Kampstra, N. A. *et al.* Results of the standard set for pulmonary sarcoidosis: feasibility and multicentre outcomes. *ERJ Open Res.* **5**, 00094–02019 (2019).

21. King, T. E. *et al.* A Phase 3 Trial of Pirfenidone in Patients with Idiopathic Pulmonary Fibrosis. *N. Engl. J. Med.* **370**, 2083–2092 (2014).

22. Faverio, P. *et al.* Progressive Fibrosing Interstitial Lung Diseases: Prevalence and Characterization in Two Italian Referral Centers. *Respiration.* **99**, 838–845 (2020).

23. Behr, J. *et al.* Survival and course of lung function in the presence or absence of antifibrotic treatment in patients with idiopathic pulmonary fibrosis: long-term results of the INSIGHTS-IPF registry. *Eur. Respir. J.* **56**, (2020).

24. Lederer, D. J. *et al.* Six-minute-walk distance predicts waiting list survival in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* **174**, 659–664 (2006).

25. Loveman, E. *et al.* The effectiveness and cost-effectiveness of treatments for idiopathic pulmonary fibrosis: systematic review, network meta-analysis and health economic evaluation. *BMC Pharmacol. Toxicol.* **15**, (2014).

26. Gangemi, A. J. *et al.* Mortality for sarcoidosis patients on the transplant wait list in the Lung Allocation Score era: Experience from a high volume center. *Respir. Med.* **157**, 69–76 (2019).

27. Juge, P.-A. *et al.* MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease . *N. Engl. J. Med.* **379**, 2209–2219 (2018).

28. Stock, C. J. *et al.* Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax* **68**, 436–441 (2013).

29. Tiosano, S. *et al.* The long-term prognostic significance of sarcoidosis-associated pulmonary hypertension – A cohort study. *Clin. Immunol.* **199**, 57–61 (2019).

30. Handa, T. *et al.* Incidence of pulmonary hypertension and its clinical relevance in patients with sarcoidosis. *Chest* **129**, 1246–1252 (2006).

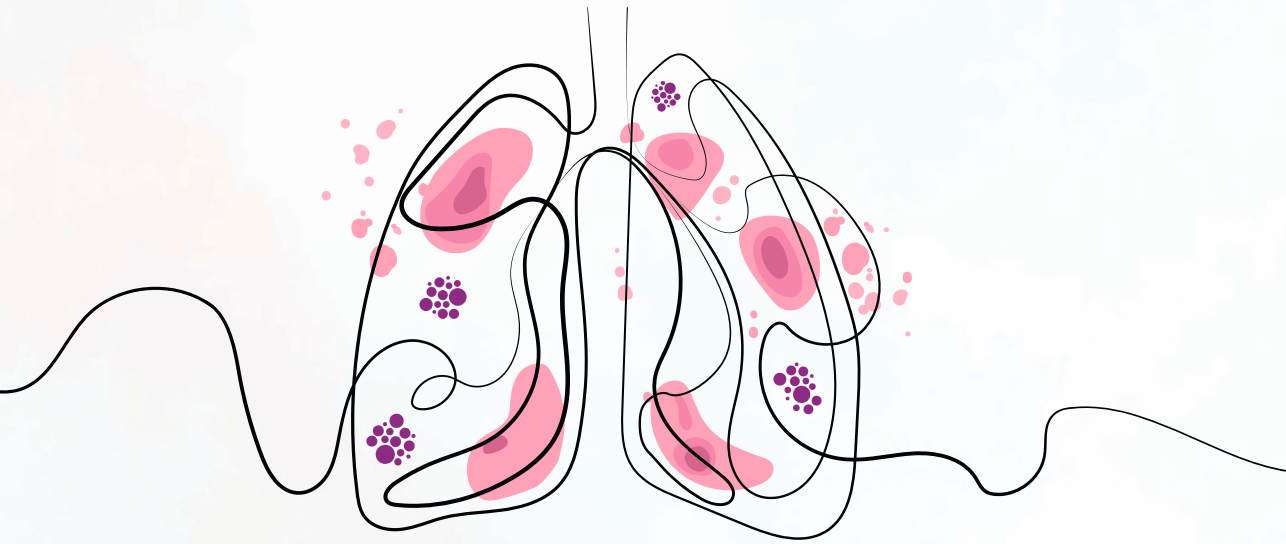
31. Huitema, M. P. *et al.* Prevalence of pulmonary hypertension in pulmonary sarcoidosis: the first large European prospective study. *The European respiratory journal* vol. 54 (2019).

32. Bourbonnais, J. M. & Samavati, L. Clinical predictors of pulmonary hypertension in sarcoidosis. *Eur. Respir. J.* **32**, 296–302 (2008).

33. Alhamad, E. H., Idrees, M. M., Alanezi, M. O., Alboukai, A. A. & Shaik, S. A. Sarcoidosis-associated pulmonary hypertension: Clinical features and outcomes in Arab patients. *Ann. Thorac. Med.* **5**, 86–91 (2010).

34. Milman, N., Svendsen, C. B., Iversen, M., Videbæk, R. & Carlsen, J. Sarcoidosis-associated pulmonary hypertension: Acute vasoresponsiveness to inhaled nitric oxide and the relation to long-term effect of sildenafil. *Clin. Respir. J.* **3**, 207–213 (2009).

35. Sulica, R. *et al.* Distinctive clinical, radiographic, and functional characteristics of patients with sarcoidosis-related pulmonary hypertension. *Chest* **128**, 1483–1489 (2005).



CHAPTER 8

SUMMARY AND GENERAL DISCUSSION

This thesis aimed to further define the role of existing biomarkers and to identify new biomarkers in different phenotypes of patients with sarcoidosis. Sarcoidosis is a difficult diagnosis due to heterogeneous clinical presentations and a lack of sensitive and specific diagnostic tests[1,2]. Also, the disease course is unpredictable, varying from spontaneous resolution to a progressive disease course with pulmonary fibrosis[3]. The above mentioned reasons make management of sarcoidosis challenging. Enhanced understanding of biomarkers and phenotypes could help clinicians to guide patients regarding diagnosing, prognostication and treatment decisions.

CHAPTER 2

In this chapter, an overview is provided of the conventional serological and bronchoalveolar lavage (BAL) markers that are most studied and clinically used in sarcoidosis. Today, still the most common used serological biomarker in sarcoidosis is angiotensin converting enzyme (ACE). However, up till now studies have shown that ACE has limited diagnostic sensitivity and also limited specificity as increased levels have been reported in other diseases. Additionally, measured levels of ACE can be misinterpreted when information on the I/D polymorphism in the ACE-gene is not known in a particular patient or due to use of ACE-inhibitors. Next to ACE, the serological biomarker soluble interleukin 2 receptor (sIL-2R) is increasingly used in the daily clinical practice of sarcoidosis. However, studies on the diagnostic value of sIL-2R with a control group are scarce and mostly outdated. In addition to serological markers, BAL serves as a valuable tool in the diagnosis of sarcoidosis. The presence of lymphocytosis and an increased CD4+/CD8+ ratio (>3.5) enhances the probability of sarcoidosis.

Since the publication of our overview, a range of new reviews on biomarkers in sarcoidosis have been published[1,2]. Unfortunately, none of these recent publications revealed ground-breaking developments in the field of biomarkers in sarcoidosis. Nevertheless, the last decade several exciting new biomarkers have been studied. Especially, promising results have been reported on the use of omics in sarcoidosis[4]. This new technique profiles sets of molecules in order to unravel potential biomarkers. Omics technology can be used in risk stratification of patients with sarcoidosis and create new biomarkers for precision medicine.

As mentioned above, we found a paucity of studies on sIL-2R in sarcoidosis with a control group. Therefore, we performed a study on the diagnostic value of sIL-2R with a control group with healthy subjects and with other interstitial lung diseases (ILD). The results of this study will be discussed in the next paragraph.

CHAPTER 3

In this chapter we provided data on the diagnostic and prognostic value of serum sIL-2R in sarcoidosis compared to healthy controls and patients with other ILD. Sensitivity of sIL-2R for the diagnosis sarcoidosis was 90%, when using a cut-off value of 3000pg/ml. Our data showed that sIL-2R is not specific for sarcoidosis, as it is also increased in idiopathic pulmonary fibrosis (IPF) and chronic hypersensitivity pneumonitis (cHP). Receiver operating curve revealed that sIL-2R level higher than 2300pg/ml could differentiate sarcoidosis from healthy subjects with a sensitivity of 95% and a specificity of 100%. However, the diagnostic accuracy significantly decreases when the control group comprises patients with other ILD. For example, only sIL-2R levels higher than 5200pg/ml could differentiate sarcoidosis from cHP and IPF with a sensitivity of 53% and a specificity of 79%. In the second part of our study on sIL-2R in sarcoidosis we showed that sIL-2R is a valuable prognostic marker for chronicity in sarcoidosis.

Since the publication of our data, several new reports have been published on the use of sIL-2R in sarcoidosis. The sensitivity of sIL-2R described in these recent studies varied between 47% and 94%[5–7], and the specificity varied between 85% and 100%[5,8]. The specificity in literature is higher than the specificity of 79% found in our study. A possible explanation for the lower specificity in our study may be found in the following: sIL-2R is produced by activated lymphocytes[9], and because lymphocytes are involved in many diseases it is not specific for the diagnosis sarcoidosis. The wide variety of reported sensitivity and specificity values described in literature can be attributed to differences in used cut-off values and control groups. As shown in our cohort, the diagnostic accuracy of sIL-2R significantly increased when the control group included healthy controls in comparison to a control group with patients with other ILD.

However, our control group consisted of healthy subjects and patients with other ILD, which is a limitation. Ideally, a prospective study on the diagnostic utility of sIL-2R should be conducted including patients that are referred to the hospital with suspected sarcoidosis. Such a study design would enable to form a control group with patients with suspected sarcoidosis that eventually did not have the disease. Another limitation is that the cohort used is relatively small, which might have caused further imprecision in the estimated diagnostic accuracy of sIL-2R. Gupta et al. performed a meta-analysis evaluating the diagnostic utility of sIL-2R and selected 10 studies comprising 592 patients[10]. This meta-analysis demonstrated a pooled sensitivity of sIL-2R of 88% and a pooled specificity of 87% for the diagnosis of sarcoidosis[10], which is higher than the sensitivity and specificity found in our cohort. As sarcoidosis is a rare disease, conducting a study with a large sample size can be challenging. This may be overcome

by performing a multicentre study. However, performing a multicentre study to the diagnostic value of sIL-2R will also create challenges e.g. due to variability of methods used in the laboratories.

Finally, our observations concerning the prognostic value of sIL-2R are in line with the results of other recent studies on the prognostic value of sIL-2R in sarcoidosis. Zhou et al.[11] found that sIL-2R was an independent predictor of spontaneous remission, with lower levels of sIL-2R in patients with spontaneous remission. Another study on the prognostic value of markers in pulmonary sarcoidosis demonstrated significantly higher levels of sIL-2R in patients with parenchymal infiltration, suggesting that sIL-2R reflects the lymphocytic alveolitis in sarcoidosis[12].

Taken together, given the limited specificity of sIL-2R, we advocate for the utilisation of sIL-2R as diagnostic biomarker when combined with other biomarkers or in the context of supportive clinical findings. In addition, as prognostic marker sIL-2R might help in the guidance of therapeutic decision making next to other biomarkers. As sarcoidosis is a complex multisystem disease one serological biomarker cannot capture the complexity of the disease nor reflect the extension of the disease as nearly every organ can be involved. In this field, Walsh et. al. did some interesting work. Their group has developed a prognostic algorithm including high-resolution computed tomography (HRCT) variables and lung function[13]. Regrettably, sensitivity and specificity rates of this algorithm were not reported; only Hazard ratio (which is an indication of risk of mortality) turned out to be 4.9-5.5. The use of another evaluation ratio complicates comparison. Another limitation of their algorithm is that, this tool is only applicable in patients with pulmonary sarcoidosis. In our opinion, a prognostic algorithm including sIL-2R and other biomarkers that is applicable in both pulmonary as well as extra-pulmonary sarcoidosis would be preferable and probably most useful in future clinical management.

CHAPTER 4

We herein discussed the results of our study on the use of human leukocyte antigen (HLA) tag single nucleotide polymorphisms (SNPs) in sarcoidosis. In the first part of this study, we validated tag SNPs rs2040410 and rs3135388 for *HLA-DRB1**0301 and *HLA-DRB1**1501 in sarcoidosis. We demonstrated that the tag SNPs completely overlapped with the corresponding *HLA-DRB1* alleles. In the second part of this study, we associated the BAL phenotypes with different HLA genotypes in Löfgren's syndrome (LS) and non-LS sarcoidosis patients. In the whole group, LS and non-LS, the CD4+/CD8+ ratio

was higher in *03+/*15- patients compared to *03–/*15+ patients. Furthermore, in LS patients the *03-/*15+ patients had significantly higher lymphocyte percentages compared to *03+/*15- patients.

HLA genotypes have been linked to the incidence and prognosis of sarcoidosis. In addition, *HLA-DRB1**0301 is associated with LS and also with a favourable prognosis. More specifically, 95% of the LS patients with *HLA-DRB1**0301 have resolving disease within two years[14]. On the other hand, *HLA-DRB1**1501 is associated with chronic course of sarcoidosis[15]. With our study we demonstrated that tag SNPs can be used to avoid the laborious analysis of HLA genotyping. Thus far, the use of the tag SNPs rs2040410 and rs3135388 had only been investigated in other immune disorders, such as diabetes mellitus type I and multiple sclerosis[16,17], but not in sarcoidosis.

In the second part of the study, we demonstrated that tag SNPs for *HLA-DRB1**0301 and -*DRB1**1501 correlated with BAL cell phenotypes. Previous studies concerning the correlation between BAL cell phenotypes and different HLA types are in line with the results of our study. Planck et al., reported a significant lower lymphocyte percentage and increased CD4+/CD8+ ratio in *03+ patients as well[18]. Idali and colleagues also found a trend of lower lymphocyte percentage in *03+ patients, however, this did not reach statistical significance[19]. Taken all together, this demonstrates that *03+ patients have lower lymphocyte percentages and higher CD4+/CD8+ ratio than *03- patients.

Therefore, it seems that higher lymphocyte percentage is associated with a less favourable prognosis and might be used to consider indication of early initiation of treatment.

Building upon these insights, a recent Swedish study found a correlation between gene expression signatures via expression quantitative trait loci (eQTLs) and BAL cell populations in sarcoidosis. In this study it was suggested that these different gene variants modulate the BAL cell types, thereby contributing to a deeper understanding of the complex interplay between genetics and BAL cell phenotypes[20].

Although our study was the first to report on the usefulness of these tag SNPs in sarcoidosis, it also has limitations. Foremost, this study had some missing data due to the retrospective study design. This led to small numbers of patients in the different subgroups which hindered comparison between groups. However, despite the small sizes of the different subgroups we were still able to demonstrate significant differences between the different HLA tag SNPs and BAL phenotypes.

In conclusion, we suggest the use of the tag SNPs rs2040410 and rs3135388 to be incorporated in daily clinical practice next to other prognostic markers, such as sIL-2R, to distinguish patients with a good prognosis from the patients with an unfavourable prognosis. Based on our study results, we recommend that patients positive for *HLA-DRB1**1501 should have more frequent regular controls enabling early interventions in case of deterioration, whereas patients positive for *HLA-DRB1**0301 could safely benefit from a wait and see policy.

CHAPTER 5

In this chapter we compared SUVmax to total lung glycolysis (TLuG), a new semiquantitative value of 18F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) describing the cumulative metabolic activity of the lung parenchyma, as therapeutic biomarkers in patients with pulmonary sarcoidosis treated with infliximab. Change in SUVmax correlated significantly with change in TLuG during infliximab therapy. In addition, SUVmax and TLuG correlated equally with lung function change during infliximab therapy and performed equally in predicting lung function change after treatment. In other words, TLuG was not superior to SUVmax in determining and predicting lung function response to infliximab in patients with pulmonary sarcoidosis. Since TLuG is a time-consuming tool, based on the fact that the segmentation of the total lung volume was adjusted manually, we recommend to use SUVmax over TLuG in determining therapeutic response in patients with pulmonary sarcoidosis.

Recently, several other interesting papers have been published in the field of quantitative measurement of ^{18}F-FDG PET/CT. First of all, Papiris and co-workers[21] studied the total lesion glycolysis (TLG) (metabolic volume x SUVmean) in pulmonary sarcoidosis as marker of the whole burden of lung inflammation in sarcoidosis and found no association between lung function indices and TLG.

Also in cardiac sarcoidosis, a similar method to assess inflammatory activity of the heart has been studied: the cardiac metabolic activity. The total cardiac metabolic activity (tCMA) is the product of the SUVmean and the cardiac metabolic volume, a measurement of the myocardium volume with high metabolism within a given boundary using a threshold[22]. High tCMA is predictive for cardiac events in patients with suspected cardiac sarcoidosis. Therefore, tCMA seems a promising method for prognostication in cardiac sarcoidosis.

Another example of a novel quantitative SUV-based quantitative measure examined in patients with cardiac sarcoidosis is the uptake index (UI) introduced by Subramanian and colleagues[23]. The UI is defined as the product of the SUVmax and the number of left ventricular segments with an abnormal uptake. The UI was a predictor of clinical and echocardiographic response after immunosuppressive therapy. In conclusion, UI seems a useful predictive biomarker.

From these studies it may be concluded that in cardiac sarcoidosis assessment of the total organ inflammatory activity is a valuable prognostic marker, whereas in pulmonary sarcoidosis assessment of the total inflammatory activity of the lungs seems not superior to SUVmax in predicting lung functional response. However, sarcoid granuloma often locate along perilymphatic regions relatively sparing the lung parenchyma and the lung function[24]. This might explain why lung function indices only weakly correlate with the burden of inflammatory activity on ^{18}F-FDG PET. Therefore, lung function alone may not be the most adequate reflection of treatment response in sarcoidosis.

A future study investigating the use of artificial intelligence (AI) in the field of biomarkers in sarcoidosis would be most interesting. The last view years promising data have been published on the use of radiomics in interstitial lung diseases, a high-throughput method that enables the extraction of vast quantities of data from images and facilitating the characterisation of the phenotype of the lesion[25].

One example of the use of AI in ILD is the application of deep learning analysis for the identification of morphological ILD patterns on HRCT[26–28]. In this context, the use of AI has also been explored in the assessment of ^{18}F-FDG PET/CT in sarcoidosis. One study demonstrated that radiomics signatures could differentiate sarcoidosis from Hodgkin lymphoma and diffuse large B-cell lymphoma equal to or better than physicians[29]. Another direction of studies for future research in the field of imaging biomarkers might be the use of fibroblast activation protein-inhibitor PET/CT (FAPI PET/CT). The currently used imaging modalities struggle to differentiate between ongoing inflammation and strictly fibrosis in sarcoidosis. FAPI PET/CT might play a potential role in this differentiation. Targeting of fibroblast activation protein inhibitors by radioactive tracer molecules can picture activated fibroblasts on FAPI PET/CT[30]. A few case reports have been published on the use of FAPI PET/CT in sarcoidosis[31–33]. Future research should explore the potential role of this promising new imaging modality in sarcoidosis.

All in all, new techniques in the field of quantitative measurement are promising and could further improve the management of sarcoidosis significantly in the upcoming years.

CHAPTER 6

We herein described the efficacy and safety of the infliximab biosimilar Inflectra® in patients with severe sarcoidosis. High medical costs of tumour necrosis factor alpha (TNF-α) inhibitors have led to worldwide inequities in accessibility to this effective third-line treatment of sarcoidosis[34]. The availability of the more affordable biosimilars has emerged a potential solution. Our study results showed that infliximab biosimilar Inflectra® seems effective in the treatment of refractory sarcoidosis, as lung function and health-related quality of life significantly improved, and sIL-2R and SUVmax significantly decreased after treatment. Furthermore, our results demonstrated that infliximab biosimilar Inflectra® is safe. Hospitalisation due to infections occurred in four patients, however after recovery all patients reinitiated Inflectra®. In addition, all patients had detectable trough levels, which indicates that there was no formation of antibodies towards Inflectra®.

Since the publication of our study, a few new reports have been published in the field of biosimilars in sarcoidosis. A small retrospective study including twenty patients demonstrated that infliximab biosimilar was effective and safe in the treatment of neurosarcoidosis[35]. Another study demonstrated that switching from originator infliximab Remicade® or biosimilar infliximab Inflectra® to biosimilar infliximab Flixabi® did not result in treatment discontinuation or loss of remission[36]. So, these results are completely in line with our results.

Our study has some limitations. First of all, due to the retrospective study design missing data had been inevitable. Second, the sample size of our cohort was relatively small, with only 29 patients included. However, we performed a power analysis to ensure that our sample size was sufficient to detect the same response in lung function improvement as published in the study of Vorselaars et al[37]. Power analysis had demonstrated that to detect 6.6% improvement of FVC a sample size of 15 patients was needed. Among the 29 patients included in our study, 15 patients had a pulmonary treatment indication, which was calculated to be sufficient. Finally, studies on drugs in sarcoidosis are hampered by a lack of standardised clinical endpoints. In most large drug trials in ILD FVC is taken as first outcome measure. However, as sarcoidosis is a multisystem disease and most sarcoidosis patients, even with severe pulmonary disease, tend to have a stable lung function, we have chosen for a composite score as described before in the study of Vorselaars et al.[37].

On the other hand, to our knowledge, this was the first report on the use of biosimilars in sarcoidosis. Hopefully, our results on efficacy and safety of this biosimilar of

TNF-α inhibitors may contribute to the increase of the worldwide accessibility to this treatment in sarcoidosis. According to the new ERS guidelines on treatment of sarcoidosis of 2021, TNF-α inhibitors are recommended in pulmonary sarcoidosis as third-line treatment in patients with persistent disease or relapse after second-line treatment with, for example, methotrexate or azathioprine[38]. However, high quality studies investigating the timing of initiation of therapy and switching to second-line or third-line therapy are still lacking. In rheumatoid arthritis, unlike in sarcoidosis, pharmacological strategy is aimed at early initiation of therapy, directly after diagnosis, with disease-modifying antirheumatic drugs (DMARDs) and therapy should be changed if there is no improvement by three months or if the target has not been reached by 6 months[39]. However, this pharmacological strategy might be too aggressive in sarcoidosis patients as useful prognostic biomarkers are still limited and as most patients with advanced pulmonary sarcoidosis do not suffer from progressive lung function loss as we demonstrated in chapter 7. Future research could further examine different approaches of timing of initiation of second- and third-line treatment in various sarcoidosis phenotypes.

CHAPTER 7

This chapter described the characteristics of a cohort with patients with advanced pulmonary sarcoidosis and provided insights in the progressive fibrosing phenotype. In our cohort with advanced pulmonary sarcoidosis, defined as patients with a diffusing capacity of the lung for carbon monoxide (DLCO) of less than 50%, evolution of lung function varied widely in the two years of follow-up. Interestingly, the majority of the patients had a stable or improved forced vital capacity (FVC) after two years of follow-up while their DLCO was severely limited. Multivariate analysis demonstrated that independent predictors of mortality and lung transplantation were: presence of pulmonary hypertension (PH), usual interstitial pneumonia (UIP)-like pattern on HRCT, and progressive fibrosing ILD (PF-ILD). In addition, 15% of our cohort with advanced pulmonary sarcoidosis met the criteria of PF-ILD according to the criteria described in the INBUILD trial[40]. Although this phenotype seems rare in sarcoidosis, our results also demonstrated that PF-ILD is a lethal phenotype with a high 10-year mortality of 43% compared to a 10-year mortality of 13% in the non-PF-ILD group. The last observation in this study was the fact that three out of the four sarcoidosis patients with a UIP-like pattern carried the Mucin 5 B (*MUC5B)* T-allele[41].

In literature, several studies reported on predictors of mortality in sarcoidosis[13,42–46]. The finding that PH is a predictor of mortality is in accordance with previous studies

concerning mortality in sarcoidosis[42,43,46,47]. Furthermore, several studies have identified extensive fibrosis on HRCT as predictor of mortality. However, none of the studies investigated a UIP-like pattern as predictor of mortality. Although this UIP-like pattern is rare in sarcoidosis[48], our study results demonstrated that this pattern is an important predictor of mortality. In addition, we found that three out of the four patients with a UIP-like pattern carried *MUC5B* T-allele[41]. The presence of the *MUC5B* minor T-allele predisposes for IPF, in which the presence of a UIP pattern is one of the hallmarks of this disease[49]. The presence of the *MUC5B* minor T-allele also predisposes for a UIP pattern in patients with RA-ILD and is associated with fibrosis in cHP patients[50,51]. In pulmonary sarcoidosis, no association was found between fibrotic pulmonary sarcoidosis and presence of the *MUC5B* minor T-allele[52]. However, in fibrotic pulmonary sarcoidosis with a UIP pattern the significance of *MUC5B* is yet unknown. Even though the number of patients with a UIP-like pattern in our cohort was too small to draw a firm conclusion, we consider *MUCB5* as a potentially important factor correlated to a UIP-like pattern in patients with fibrotic pulmonary sarcoidosis. In IPF, *MUC5B* promotor polymorphism predisposes for disease[49,53,54], however, it has also been shown that carriage of this *MUC5B* promotor polymorphism in patients with IPF is associated with an improved outcome[55]. The association between *MUC5B* T allele and sarcoidosis patients with a UIP-like pattern should be studied. In addition, an intriguing direction for future research would be to explore the prognostic role of *MUCB5* promotor polymorphism in this specific subgroup of sarcoidosis.

Another predictor of mortality in sarcoidosis that has been described in literature is a composite physiological index (CPI) above 40[13,42]. Our study, however, did not identify CPI as a significant predictor of mortality. A possible explanation for this might be that more than 80% of our patients had a CPI above 40 due to our inclusion criteria of a DLCO of less than 50% of predicted. Consequently , our study was impeded to establish CPI as predictor in this context.

The prevalence of PF-ILD in the sarcoidosis population has been described in only a few other studies[48,56]. In one study, including 32 patients with sarcoidosis with Scadding stage IV, 41% met the criteria of progression of the INBUILD trial[56]. The prevalence of PF-ILD of 41% of the patients with Scadding stage IV, is much higher than the prevalence of PF-ILD of 15% found in our cohort. However, we think that this may be an overestimation of the prevalence of PF-ILD, because the criterion of 10% of fibrosis of the lungs was not applied in this study. Instead, all patients with Scadding stage IV that showed progression were labelled as PF-ILD, which might explain the large gap in found prevalence.

With respect to the epidemiology of PF-ILD an interesting study has been published recently. This large multicentre retrospective study demonstrated that among the non-IPF fibrosing ILD, sarcoidosis was the most common disease in most countries. In addition, 11% of the sarcoidosis patients had PF-ILD and 2% had PF-ILD and a UIP-like pattern[48].

Lastly, our study results are important in the light of treatment of PF-ILD. Over the last few years there has been an increasing interest in the use of antifibrotics in sarcoidosis patients with pulmonary progressive fibrosis. The INBUILD trial, a large randomised controlled trial, demonstrated that nintedanib slowed lung function decline in patients with PF-ILD other than IPF, including a few patients with sarcoidosis[40]. Nowadays, the phenotype of progressive fibrosis in patients with ILD is called progressive pulmonary fibrosis (PPF). An important difference between PPF and PF-ILD is the fact that time to progression in PPF is decreased to one year, in contrast to time of progression over two years in the former PF-ILD criteria. Recently, a consensus statement on PPF has been published[57]. This statement dissuades standardised management regiment that can be applied to all patients with PPF because of the variability in rate of progression emphasising case-by-case decisions. This statement underlines the importance of an accurate initial diagnosis as this is associated with the risk of progression. It is debatable that an exception to this case-by-case approach should be made for patients with a UIP-like pattern with a known cause (e.g. HP or connective tissue disease-associated ILD) . Some researchers argue that all patients with UIP, thus patients with IPF and patients with UIP with a known cause, should be viewed as single diagnostic entity[58], as they appear to have a comparable rate of progression of lung function decline[40,59]. In addition, our data together with other studies[60,61] demonstrated that UIP-like pattern is an important risk factor in patients with other fibrotic ILD.

Taken all together, one could argue that sarcoidosis patients with a UIP-like pattern should be treated upfront with antifibrotics. However, longitudinal data on patients with sarcoidosis with a UIP-like pattern are scarce given the rarity of a UIP-like pattern in sarcoidosis. Therefore, we are careful to draw firm conclusions and we agree with the recommendation of the statement on PPF that the decision on the use of antifibrotics cannot be standardised and should be made case-by-case.

Longitudinal data on sarcoidosis patients with PPF are warranted to gain insights in the course of disease and rate of progression in this phenotype.

An obstacle in our study is the ambiguous terminology used in literature for advanced sarcoidosis. In absence of a clear definition of advanced pulmonary sarcoidosis we used

the inclusion criterion of DLCO of less than 50% of predicted. However, given this specific inclusion criterion we are careful to extrapolate our results to the whole sarcoidosis population. Another limitation is that this study was conducted retrospectively, confronting us with lung function and HRCT data that were not performed at strictly set times which resulted in missing data. A future study should include a larger cohort with patients with PPF which will illuminate characteristics and predictors of the prognosis of PPF in sarcoidosis.

In conclusion, the results in this chapter demonstrated that PF-ILD is a relatively rare but lethal phenotype in sarcoidosis. Therefore, further insights in this phenotype are urgently needed to clarify the therapeutic strategy in this patient group to prevent overtreatment but minimise the change of undertreatment with antifibrotic therapy.

FUTURE PERSPECTIVES

The research described in the present thesis aimed to provide new insights in known and novel biomarkers in different phenotypes of sarcoidosis, helping their possible implementation in everyday clinical management of patients. Figure 1 shows a multidimensional pictogram of the biomarkers studied and their potential usefulness in light of diagnosis, monitoring as well as prognostication in this heterogeneous disease.

The final paragraph of this thesis will provide some perspectives on future research in the field of biomarkers in sarcoidosis.

Diagnostic algorithms

Future studies are needed to build and validate a diagnostic algorithm with a panel of serological biomarkers. Sarcoidosis is a complex disease with a largely unresolved aetiology and therefore the quest for the ideal single biomarker seems unrealistic. It seems more plausible that a combination of biomarkers will perform better in diagnosing and prognosing sarcoidosis than a single biomarker. Furthermore, the clinical context will also influence the choice of biomarker(s) used to address a clinical question such as diagnosis, monitoring disease activity or monitoring response to treatment. Regarding the diagnosis of sarcoidosis, combining different biomarkers can be useful. Ishihara et al.[62] found that specificity of ACE was higher than sIL-2R (100% vs 93%), but sensitivity of sIL-2R for detection of sarcoidosis was higher (44% vs 69%) than ACE. Combining the two biomarkers, the sensitivity and specificity of elevated sIL-2R and/or ACE were 75% and 93% respectively, and therefore concluded that serum sIL-2R in addition to ACE levels improved the sensitivity in the detection of uveitis associated

with sarcoidosis[62]. Another study demonstrated that both sensitivity and specificity regarding the diagnosis of sarcoidosis increased when results of ACE and chitotriosidase were combined[63]. Taken all together, the development and standardisation of a panel of multiple biomarkers could improve the diagnostic process in sarcoidosis.

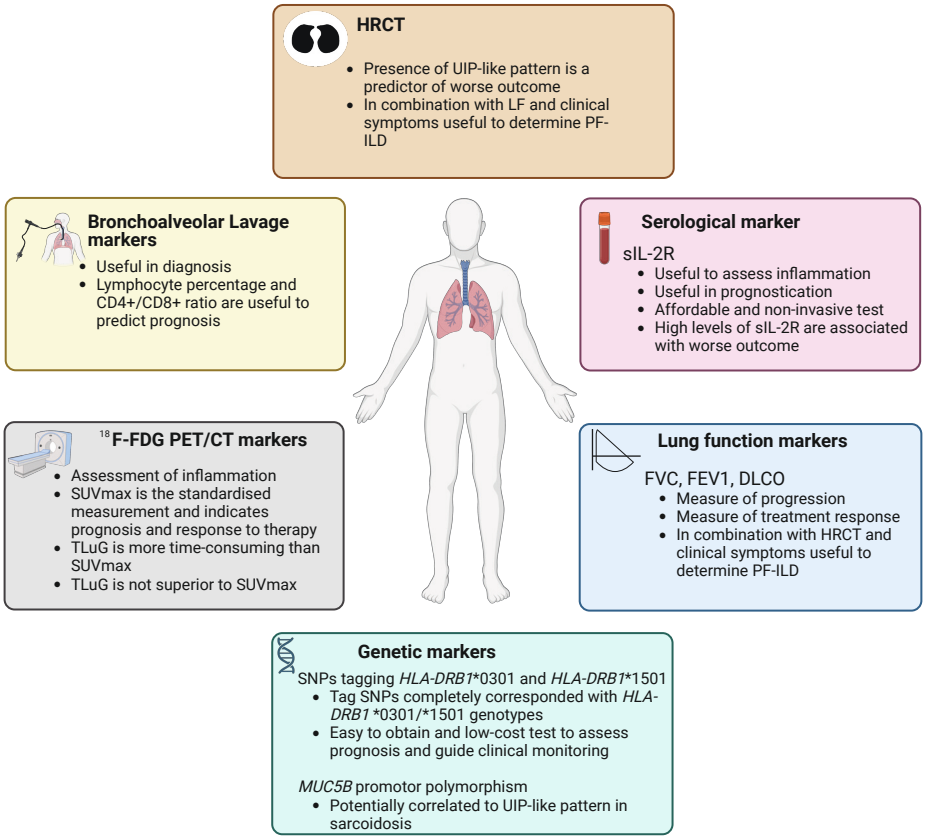


Figure 1. Schematic overview of different biomarkers studied in this thesis and possible implications in daily clinical practice.
HRCT= high-resolution computed tomography; UIP= usual interstitial pneumonia; LF = lung function; PF-ILD= progressive pulmonary fibrosis; sIL-2R= soluble interleukin 2 receptor; ^{18}F-FDG PET/CT= fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis; FVC= forced viral capacity; FEV1= forced expiratory volume in one second; DLCO= diffusing capacity of the lungs for carbon monoxide; SNP= single nucleotide polymorphism; *MUC5B*= Mucin 5B.

Sarcoidosis in primary care

As a general physician in training, my view on sarcoidosis care in the Netherlands has shifted. As our health care system is under pressure of rising health care costs, a

solution might be found in the transition of suitable non-complex and low-risk patients from secondary care to primary care, which might lead to considerable cost savings. In the Netherlands, the follow-up of sarcoidosis patients takes place in secondary care in general. We suggest that after diagnosis follow-up of sarcoidosis patients can take place in primary care in a carefully selected subset of patients with a good prognosis, low-risk patients. Improving risk stratification with prognostic markers in sarcoidosis patients can facilitate the shift to primary care. A combination of a disease presentation associated with a good prognosis, i.e. acute onset of disease and the typical triad of Löfgren's syndrome, together with prognostic markers should contribute to the decision to continue the follow-up of sarcoidosis in primary care. A prognostic algorithm should be built and validated to select low-risk patients. The following prognostic markers can contribute to this algorithm: tag SNPs for *HLA-DRB1**0301 and *HLA-DRB1**1501, sIL-2R, percentage of BAL lymphocytes, and CD4+/CD8+ ratio in BAL. In line with this view, Grunewald et al. demonstrated that 95% of the patients with Löfgren's syndrome and positive for *HLA-DRB1**03 had resolving disease within two years[14].

If a part of the sarcoidosis care is transitioned to the primary care we have a few recommendations. First, a prognostic algorithm, based on clinical picture and prognostic markers, should be built to carefully select patients with a low-risk profile. Second, patients should be educated to seek medical help in case of worsening of symptoms or the presence of new symptoms. Third, low-threshold communication should be provided by pulmonologists for consultation and timely referral in case of worsening of the disease. Finally, up-to-date information on sarcoidosis should be accessible for primary caregivers.

CONCLUSION

To conclude, in a heterogeneous disease like sarcoidosis validated biomarkers enabling personalised medicine is an unmet clinical need of high importance. The data presented in this thesis provide novel insights regarding the use of current as well as novel biomarkers in management of patients with various phenotypes of sarcoidosis. It will hopefully stimulate further research into the role of these biomarkers, helping the field to take another step into personalised medicine in patients with this multifaceted disease.

REFERENCES

1. Trisolini R, Spagnolo P, Baughman, R. Principles of diagnosis. in *Sarcoidosis (ERS Monograph)* 57–74 (2022).
2. Korenromp, I. H. E., Maier, L. A. & Grutters, J. C. Serum and imaging biomarkers. in *Sarcoidosis (ERS Monograph)* 107–121 (2022).
3. Baughman, R. P. *et al.* Clinical Characteristics of Patients in a Case Control Study of Sarcoidosis. *Am J Respir Crit Care Med* **164**, 1885–1889 (2001).
4. Crouser, E. D. *et al.* Application of "omics" and systems biology to sarcoidosis research. *atsjournals.org* **14**, 445–451 (2017).
5. Eurelings, L. E. M. *et al.* Sensitivity and specificity of serum soluble interleukin-2 receptor for diagnosing sarcoidosis in a population of patients suspected of sarcoidosis. *PLoS One* **14**, e0223897 (2019).
6. Thi Hong Nguyen, C., Kambe, N., Kishimoto, I., Ueda-Hayakawa, I. & Okamoto, H. Serum soluble interleukin-2 receptor level is more sensitive than angiotensin-converting enzyme or lysozyme for diagnosis of sarcoidosis and may be a marker of multiple organ involvement. *J. Dermatol.* **44**, 789–797 (2017).
7. Miyata, J. *et al.* Serum soluble interleukin-2 receptor level is a predictive marker for EBUS-TBNA-based diagnosis of sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **37**, 8–16 (2020).
8. Uysal, P. *et al.* YKL-40, Soluble IL-2 Receptor, Angiotensin Converting Enzyme and C-Reactive Protein: Comparison of Markers of Sarcoidosis Activity. *Biomolecules* **8**, 10.3390/biom8030084 (2018).
9. Rubin, L. A. *et al.* Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol. (Baltimore, Md. 1950)* **135**, 3172–3177 (1985).
10. Gupta, S., Miloni Parmar, Padappayil, R. P., Bansal, A. & Daouk, S. Role of Serum Soluble Interleukin-2 Receptor Level in the Diagnosis of Sarcoidosis: A Systematic Review and Meta-Analysis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **40**, e2023005 (2023).
11. Zhou, Y. *et al.* sIL-2R levels predict the spontaneous remission in sarcoidosis. *Rspiratory Med.* **37**, 8–16 (2020).
12. Miyoshi, S. *et al.* Comparative evaluation of serum markers in pulmonary sarcoidosis. *Chest* **137**, 1391–1397 (2010).
13. Walsh, S. L. F. *et al.* An integrated clinicoradiological staging system for pulmonary sarcoidosis: A case-cohort study. *Lancet Respir. Med.* **2**, 123–130 (2014).
14. Grunewald, J. & Eklund, A. Löfgren's syndrome: Human leukocyte antigen strongly influences the disease course. *Am. J. Respir. Crit. Care Med.* **179**, 307–312 (2009).
15. Grunewald, J. *et al.* Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. *Respir. Res.* **11**, 25 (2010).
16. Barker, J. M. *et al.* Two single nucleotide polymorphisms identify the highest-risk diabetes HLA genotype: potential for rapid screening. *Diabetes* **57**, 3152–3155 (2008).

17. Hafler, D. A. *et al.* Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* **357**, 851–862 (2007).

18. Planck, A., Eklund, A. & Grunewald, J. Inflammatory BAL-fluid and serum parameters in HLA DR17 positive vs. DR17 negative patients with pulmonary sarcoidosis. *Sarcoidosis, Vasc. Diffus. lung Dis. Off. J. WASOG* **18**, 64–9 (2001).

19. Idali, F. *et al.* Reduced Th1 response in the lungs of HLA-DRB1*0301 patients with pulmonary sarcoidosis. *Eur. Respir. J.* **27**, 451–459 (2006).

20. Abo Al Hayja, M. *et al.* Functional link between sarcoidosis-associated gene variants and quantitative levels of bronchoalveolar lavage fluid cell types. *Front. Med.* **10**, 1061654 (2023).

21. Papiris, S. A. *et al.* 18F-FDG PET/CT in Pulmonary Sarcoidosis:Quantifying Inflammation by the TLG index. *Expert Rev. Respir. Med.* **14**, 103–110 (2020).

22. Tuominen, H. *et al.* FDG-PET in possible cardiac sarcoidosis: Right ventricular uptake and high total cardiac metabolic activity predict cardiovascular events. *J. Nucl. Cardiol.* **28**, 199–205 (2021).

23. Subramanian, M. *et al.* Pre-Treatment Myocardial 18 FDG Uptake Predicts Response to Immunosuppression in Patients With Cardiac Sarcoidosis. *JACC Cardiovasc Imaging.* **14**, 2008–2016 (2021).

24. Rossi, G., Cavazza, A. & Colby, T. V. Pathology of Sarcoidosis. *Clin. Rev. Allergy Immunol.* **49**, 36–44 (2015).

25. Barnes, H. *et al.* Machine learning in radiology: the new frontier in interstitial lung diseases. *Lancet Digit. Heal.* **5**, e41–e50 (2023).

26. Anthimopoulos, M., Christodoulidis, S., Ebner, L., Christe, A. & Mougiakakou, S. Lung Pattern Classification for Interstitial Lung Diseases Using a Deep Convolutional Neural Network. *IEEE Trans. Med. Imaging* **35**, 1207–1216 (2016).

27. Huang, S. *et al.* A deep convolutional neural network architecture for interstitial lung disease pattern classification. *Med. Biol. Eng. Comput.* **58**, 725–737 (2020).

28. Raju, N., Augustine, D. P. & Anita, H. B. A Novel Deep Learning Approach for Identifying Interstitial Lung Diseases from HRCT Images. *SN Comput. Sci.* **4**, (2023).

29. Lovinfosse, P. *et al.* Distinction of Lymphoma from Sarcoidosis on 18F-FDG PET/CT: Evaluation of Radiomics-Feature–Guided Machine Learning Versus Human Reader Performance. *J. Nucl. Med.* **63**, 1933–1940 (2022).

30. Röhrich, M. *et al.* Fibroblast Activation Protein-Specific PET/CT Imaging in Fibrotic Interstitial Lung Diseases and Lung Cancer: A Translational Exploratory Study. *J. Nucl. Med.* **63**, 127–133 (2022).

31. Siebermair, J., Kessler, L., Kupusovic, J., Rassaf, T. & Rischpler, C. Cardiac fibroblast activation detected by 68Gallium-FAPI-46 positron emission tomography-magnetic resonance imaging as a sign of chronic activity in cardiac sarcoidosis. *Eur. Hear. journal. Case reports* **6**, ytac005 (2022).

32. Pang, Y., Wang, P., Xu, W., Zhao, L. & Chen, H. Identification of isolated hepatic sarcoidosis with [18F]FDG and [68 Ga]Ga-FAPI PET/CT. *Eur. J. Nucl. Med. Mol. Imaging* **50**, 2918–2919 (2023).

33. Watabe, T. *et al.* Activated fibroblasts in muscle sarcoidosis revealed by [18F]FAPI-74 PET. *Eur. J. Nucl. Med. Mol. Imaging* (2023). doi:10.1007/s00259-023-06263-8

34. Putrik, P. *et al.* Inequities in access to biologic and synthetic DMARDs across 46 European countries. *Ann. Rheum. Dis.* **73**, 198–206 (2014).

35. Riller, Q. *et al.* Infliximab biosimilar for treating neurosarcoidosis: tolerance and efficacy in a retrospective study including switch from the originator and initiation of treatment. *J. Neurol.* **266**, 1073–1078 (2019).

36. Peters, B. J. M., Bhatoe, A., Vorselaars, A. D. M. & Veltkamp, M. Switching to an Infliximab Biosimilar Was Safe and Effective in Dutch Sarcoidosis Patients. *Cells* **10**, 1–8 (2021).

37. Vorselaars, A. D. *et al.* Effectiveness of infliximab in refractory FDG PET-positive sarcoidosis. *Eur. Respir. J.* **46**, 175–185 (2015).

38. Baughman, R. P. *et al.* ERS clinical practice guidelines on treatment of sarcoidosis. *Eur. Respir. J.* **58**, (2021).

39. Smolen, J. S. *et al.* EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann. Rheum. Dis.* **79**, S685–S699 (2020).

40. Flaherty, K. R. *et al.* Nintedanib in progressive fibrosing interstitial lung diseases. *N. Engl. J. Med.* **381**, 1718–1727 (2019).

41. Schimmelpennink, M. C. *et al.* Characterization of the PF-ILD phenotype in patients with advanced pulmonary sarcoidosis. *Respir. Res.* **23**, (2022).

42. Jeny, F. *et al.* Predictors of mortality in fibrosing pulmonary sarcoidosis. *Respir. Med.* **169**, 105997 (2020).

43. Nardi, A. *et al.* Stage IV sarcoidosis: Comparison of survival with the general population and causes of death. *Eur. Respir. J.* **38**, 1368–1373 (2011).

44. Gupta, R. *et al.* Predictors of lung transplant waitlist mortality for sarcoidosis ☆. *Orig. Res.* **205**, 107008 (2022).

45. Alqalyoobi, S., Liao, S. Y., Qureshi, W. & Obi, O. N. National Temporal Trends in Hospitalization and Inpatient Mortality in Patients With Pulmonary Sarcoidosis in the United States Between 2007 and 2018. *Chest* **161**, 152–168 (2022).

46. Kirkil, G., Lower, E. E. & Baughman, R. P. Predictors of Mortality in Pulmonary Sarcoidosis. *Chest* **153**, 105–113 (2018).

47. Boucly, A. *et al.* Management and long-term outcomes of sarcoidosis-associated pulmonary hypertension. *Eur. Respir. J.* **50**, (2017).

48. Hilberg, O. *et al.* Epidemiology of interstitial lung diseases and their progressive-fibrosing behaviour in six European countries. *ERJ open Res.* **8**, (2022).

49. Seibold, M. A. *et al.* A Common MUC5B Promoter Polymorphism and Pulmonary Fibrosis. *N Engl J Med.* **364**, 1503–1512 (2011).

50. Juge, P.-A. *et al.* MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease . *N. Engl. J. Med.* **379**, 2209–2219 (2018).

51. Ley, B. *et al.* The MUC5B promoter polymorphism and telomere length in patients with chronic hypersensitivity pneumonitis: an observational cohort-control study. *Lancet. Respir. Med.* **5**, 639–647 (2017).

52. Stock, C. J. *et al.* Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax* **68**, 436–441 (2013).

53. Noth, I. *et al.* Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet. Respir. Med.* **1**, 309–317 (2013).

54. Fingerlin, T. E. *et al.* Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat. Genet.* **45**, 613–620 (2013).

55. Peljto, A. L. *et al.* Association Between the MUC5B Promoter Polymorphism and Survival in Patients With Idiopathic Pulmonary Fibrosis. *JAMA* **309**, 2232–2239 (2013).

56. Faverio, P. *et al.* Progressive Fibrosing Interstitial Lung Diseases: Prevalence and Characterization in Two Italian Referral Centers. *Respiration.* **99**, 838–845 (2020).

57. Rajan, S. K. *et al.* Progressive pulmonary fibrosis: an expert group consensus statement. *Eur. Respir. J.* **61**, 2103187 (2023).

58. Selman, M., Pardo, A. & Wells, A. U. Usual interstitial pneumonia as a stand-alone diagnostic entity: the case for a paradigm shift? *Lancet. Respir. Med.* **11**, 188–196 (2023).

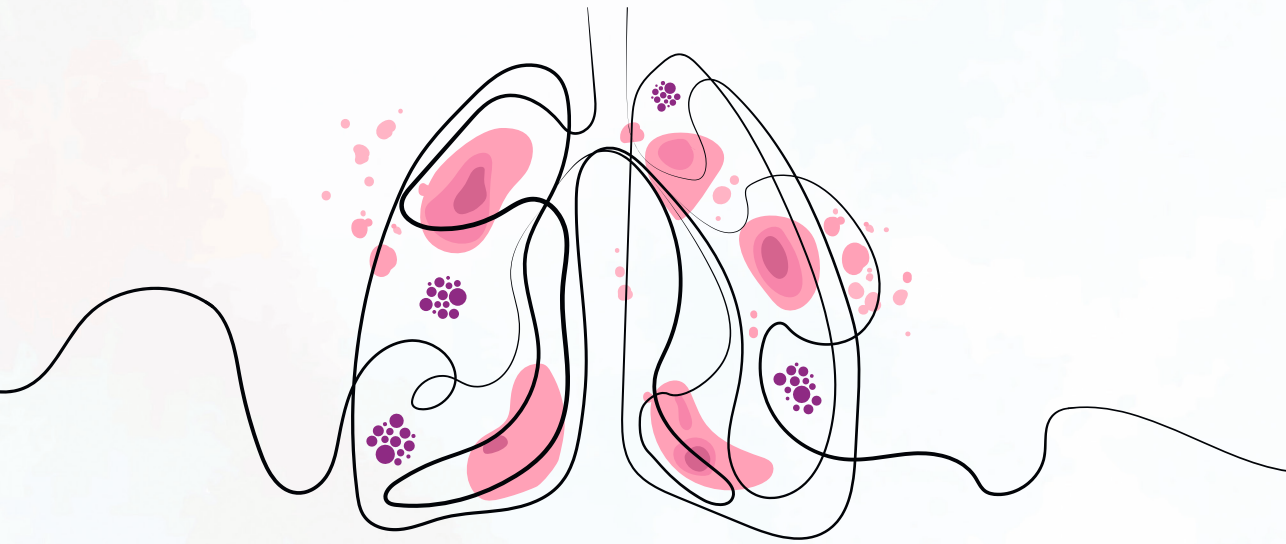
59. Strand, M. J. *et al.* Pulmonary function and survival in idiopathic vs secondary usual interstitial pneumonia. *Chest* **146**, 775–785 (2014).

60. Qiu, M. *et al.* Factors associated with mortality in rheumatoid arthritis-associated interstitial lung disease: a systematic review and meta-analysis. *Respir. Res.* **22**, (2021).

61. Wang, P. *et al.* Pathologic Findings and Prognosis in a Large Prospective Cohort of Chronic Hypersensitivity Pneumonitis. *Chest* **152**, 502–509 (2017).

62. Ishihara, M. *et al.* Usefulness of Combined Measurement of Serum Soluble IL-2R and Angiotensin-Converting Enzyme in the Detection of Uveitis Associated with Japanese Sarcoidosis. *Clin. Ophthalmol.* **14**, 2311–2317 (2020).

63. Enyedi, A. *et al.* Combined application of angiotensin converting enzyme and chitotriosidase analysis improves the laboratory diagnosis of sarcoidosis. *Clin. Chim. Acta.* **500**, 155–162 (2020).



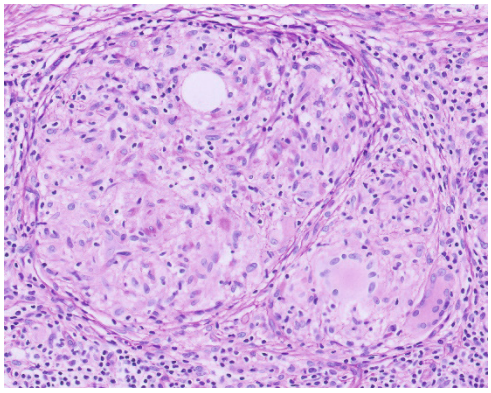
APPENDICES

NEDERLANDSE SAMENVATTING
LIST OF PUBLICATIONS
CONTRIBUTING AUTHORS
CURRICULUM VITAE
DANKWOORD

NEDERLANDSE SAMENVATTING

INTRODUCTIE

Sarcoïdose is een ontstekingsziekte die nagenoeg alle organen kan aantasten, met andere woorden een multisysteem aandoening. Echter, in het merendeel van de patiënten zijn de longen betrokken. De klinische presentatie van sarcoïdose is divers en is onder andere afhankelijk van welke organen betrokken zijn. Bovendien ervaren patiënten vaak niet-specifieke symptomen, zoals vermoeidheid. Deze wijd uiteenlopende, en vaak aspecifieke, klinische presentatie maakt sarcoïdose een lastige diagnose om te stellen. De oorzaak van sarcoïdose is tot op heden onbekend. De algemeen heersende theorie over het ontstaan ervan is dat in patiënten met een erfelijke aanleg voor sarcoïdose een bepaalde trigger leidt tot een sterke reactie van het immuunsysteem, waarbij niet-verkazende granulomen worden gevormd, dit zijn ophopingen van ontstekingscellen, zie figuur 1. De diagnose wordt gesteld als er niet-verkazende granulomen worden aangetoond, in combinatie met symptomen van sarcoïdose en als er radiologisch aanwijzingen zijn voor sarcoïdose. Sarcoïdose is een diagnose per exclusionem, dit betekent dat alle andere oorzaken voor het ontstaan van niet-verkazende granulomen, zoals tuberculose, dienen te worden uitgesloten.



Figuur 1. Biopt van een lymfeklier van een patiënt met sarcoïdose, waarbij een niet-verkazend granuloom te zien is.

Bij patiënten met sarcoïdose is het beloop van de ziekte en de prognose onvoorspelbaar. In een deel gaat de ziekte spontaan in remissie zonder behandeling. Echter, een ander deel krijgt te maken met chronische sarcoïdose, waarbij langdurig behandeling nodig kan zijn.

Het syndroom van Löfgren is een acute vorm van sarcoïdose en heeft een gunstige prognose. Het syndroom van Löfgren wordt gekenmerkt door de trias van enkelartritis, de huidafwijking erythema nodosum en vergrote lymfeklieren in de borstkas. Variatie in het gen dat codeert voor humaan leukocytenantigenen (HLA) is geassocieerd met het ontstaan en het beloop van het syndroom van Löfgren. Het *HLA-DRB1**0301-allel is geassocieerd met zowel het ontstaan van het syndroom van Löfgren als met een goede prognose. In een deel van de patiënten met sarcoïdose leidt chronische ontsteking tot het ontstaan van littekenvorming, ook wel fibrose genoemd. De aanwezigheid van fibrose in de longen is geassocieerd met een slechtere prognose in sarcoïdose. De ontwikkeling van fibrose is naast hogere leeftijd en pulmonale hypertensie, hoge bloeddruk in de longslagader, een belangrijke onafhankelijke voorspeller van de mortaliteit in sarcoïdose.

In een deel van de patiënten met sarcoïdose is behandeling noodzakelijk. Indicaties voor het starten van behandeling zijn bijvoorbeeld achteruitgang van de longfunctie of ernstige symptomen die de kwaliteit van leven beperken. De eerstelijnsbehandeling van sarcoïdose is prednison. Echter, langdurig gebruik van prednison kan ernstige bijwerkingen veroorzaken, zoals gewichtstoename, botontkalking en suikerziekte. Indien prednison niet effectief is of als patiënten ernstige bijwerkingen ervaren kan azathioprine of methotrexaat worden ingezet als tweedelijns behandeling. Een klein deel van de sarcoïdose patiënten, met bedreigende en moeilijk behandelbare sarcoïdose, wordt behandeld met derdelijns biologische medicijnen, zoals infliximab of adalimumab.

Sarcoïdose is een uitdagende diagnose door de wijd uiteenlopende klinische presentatie en het gebrek aan sensitieve en specifieke diagnostische tests. Ook voorspellen van het ziektebeloop is veelal moeilijk. Biomarkers kunnen helpen bij zowel het diagnosticeren als de risicostratificatie van sarcoïdose patiënten. Een biomarker is een indicator voor een biologisch proces, pathogeen proces of farmacologische respons, welke objectief kan worden gemeten en geëvalueerd. Deze biomarkers kunnen stofjes zijn in het bloed of in de vloeistof van een longspoeling ofwel bronchoalveolaire lavage (BAL). Echter, het kunnen ook de longfunctie-waarden zijn of bepaalde kenmerken van radiologische beelden of 18F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT). In de afgelopen decennia zijn verschillende biomarkers in sarcoïdose onderzocht, echter tot op heden is er nog geen enkele 'perfecte' biomarker gevonden.

DOEL

Het doel van deze thesis is het verbeteren van het diagnosticeren, monitoren tijdens behandeling en prognosticeren van sarcoïdose, door meer inzicht te verwerven in de rol van bestaande biomarkers en door nieuwe biomarkers te onderzoeken. Daarnaast beschrijft deze thesis nieuwe inzichten in verschillende concepten van fenotypes binnen sarcoïdose.

In **hoofdstuk 2** geven we een overzicht van bestaande biomarkers in het bloed (serum markers) en BAL markers. De volgende biomarkers worden besproken in dit hoofdstuk: angiotensin converting enzyme (ACE), serum soluble interleukin 2 receptor (sIL-2R), serum calcium en lysozym. Daarnaast worden in het tweede deel van dit hoofdstuk enkele potentiële biomarkers beschreven. De meest frequent gebruikte biomarker in sarcoïdose is ACE. Echter, ACE heeft een beperkte diagnostische sensitiviteit en specificiteit. Bovendien worden ACE-waarden soms verkeerd geïnterpreteerd, omdat I/D (insertie/deletie) polymorfismen in het ACE-gen ACE-waarden beïnvloeden, hierdoor is de normale range afhankelijk van het genotype dat de patiënt heeft. Naast ACE wordt de serum biomarker sIL-2R steeds vaker gebruikt in de dagelijkse sarcoïdose zorg. Echter, studies naar de diagnostische waarde van sIL-2R met een controlegroep zijn schaars en verouderd. Naast serologische biomarkers worden BAL biomarkers gebruikt voor het stellen van de diagnose sarcoïdose. Een verhoogd percentage lymfocyten (een type witte bloedcellen) en een toegenomen CD4+/CD8+ ratio van deze witte bloedcellen in de BAL vloeistof maakt de diagnose sarcoïdose waarschijnlijker. Sinds onze publicatie is een reeks van nieuwe reviews over biomarkers in sarcoïdose gepubliceerd. Echter, tot op heden zijn er helaas geen baanbrekende nieuwe ontwikkelingen geweest op het gebied van biomarkers in sarcoïdose. Omdat wij tijdens het schrijven van ons overzicht over biomarkers in sarcoïdose stuitten op een schaarste aan studies naar de diagnostische waarde van sIL-2R met een controlegroep, hebben wij dit verder onderzocht en beschreven in hoofdstuk 3.

In **hoofdstuk 3** beschrijven wij de diagnostische en prognostische waarde van de biomarker sIL-2R. sIL-2R wordt geproduceerd door geactiveerde T-lymfocyten en kan worden gebruikt als maat voor sarcoïdose ziekteactiviteit. In deze retrospectieve studie includeerden wij 121 patiënten met sarcoïdose (waarvan 17 patiënten met Löfgren's syndroom), 35 patiënten met chronische hypersensitiviteits pneumonitis (cHP, dit wordt tegenwoordig fibroserende HP genoemd), 62 patiënten met idiopathische pulmonale fibrose (IPF) en 70 gezonde proefpersonen. Er worden verhoogde concentraties sIL-2R gezien in zowel het bloed van sarcoïdose patiënten als van patiënten met IPF en cHP. Negentig procent van de patiënten met sarcoïdose hebben een verhoogde sIL-2R

concentratie. In patiënten met sarcoïdose worden significant hogere concentraties sIL-2R gevonden dan patiënten met cHP en IPF. Echter, hoewel patiënten met sarcoïdose de hoogste sIL-2R concentraties hebben in vergelijking tot cHP en IPF, onderscheidt sIL-2R maar matig tussen de diagnoses sarcoïdose, cHP en IPF, omdat er veel overlap is in sIL-2R concentraties. Tevens tonen onze studieresultaten dat een hoge sIL-2R concentratie voorspellend is voor een chronisch ziektebeloop in sarcoïdose met een odds ratio van 2.1. Een sIL-2R concentratie hoger dan 4700pg/mL is voorspellend voor een chronisch ziektebeloop met een sensitiviteit van 75%. Uit deze studie concluderen wij dat sIL-2R een sensitieve diagnostische biomarker is voor sarcoïdose, echter de specificiteit van deze biomarker voor de diagnose sarcoïdose is beperkt. Gezien het feit dat sarcoïdose een complexe multisysteem aandoening is, is het onwaarschijnlijk dat één serologische biomarker de complexiteit en uitgebreidheid van de ziekte kan weerspiegelen. Een panel van meerdere diagnostische biomarkers die de ziekteactiviteit, van zowel pulmonale als extra-pulmonale ziekte, weergeeft, zou uitkomst kunnen bieden voor een complex ziektebeeld als sarcoïdose. Als prognostische biomarker kan sIL-2R, in combinatie met andere prognostische biomarkers, mogelijk wel bijdragen aan het nemen van therapeutische besluiten.

In **hoofdstuk 4** valideren we het gebruik van de tag single nucleotide polymorphisms (tag SNP's) voor *HLA-DRB1**0301 en *HLA-DRB1**1501. Vervolgens evalueren we de associatie tussen deze tag SNP's en BAL fenotypes. Genetische variatie in de HLA-regio is geassocieerd met zowel de incidentie als het beloop van sarcoïdose. *HLA-DRB1**0301 is geassocieerd met het syndroom van Löfgren en met een mild ziektebeloop, namelijk 95% van de *HLA-DRB1**0301 positieve patiënten met het syndroom van Löfgren heeft spontane remissie van sarcoïdose binnen twee jaar. Daarentegen is *HLA-DRB1**1501 geassocieerd met een chronisch beloop van sarcoïdose. Het verrichten van een HLA-genotypering is kostbaar en tijdsintensief. In andere ziektes, zoals multipele sclerose en diabetes mellitus type I, is er een verband gevonden tussen het A allel van de tag SNP's rs2040410 en rs3135388 en respectievelijk *HLA-DRB1**0301 en *HLA-DRB1**1501. Het bepalen van tag SNP's is goedkoper en makkelijker dan het uitvoeren van een complexe HLA-analyse. Echter, het gebruik van deze tag SNP's rs2040410 en rs3135388 was nog niet gevalideerd voor gebruik in sarcoïdose. Onze studieresultaten tonen dat de tag SNP's volledig corresponderen met de *HLA-DRB1**0301/*1501 genotypen. In het tweede deel van onze studie onderzoeken wij de associatie tussen BAL fenotypes en de tag SNP's in zowel Löfgren als non-Löfgren patiënten. In het gehele cohort wordt een significant hoger CD4+/CD8+ ratio in de BAL gevonden in *03+/*15- patiënten in vergelijking tot *03-/*15+ patiënten en *03-/*15- patiënten. In patiënten met Löfgren's syndroom wordt een significant hoger lymfocyten percentage gevonden in *03-/*15+ patiënten in vergelijking met *03+/*15- patiënten.

Deze resultaten laten zien dat de bepaling van de tag SNP's rs2040410 en rs3135388 gebruikt kunnen worden als prognostische biomarkers. Ze kunnen naast andere prognostische biomarkers, zoals sIL-2R, nuttig zijn om de patiënten met een gunstige prognose te onderscheiden van de patiënten met een minder gunstige prognose. Bij patiënten positief voor *HLA-DRB1**1501 zou bijvoorbeeld overwogen kunnen worden om frequenter poliklinische controles aan te bieden, zodat eventuele verslechtering van het klinisch beeld sneller ontdekt kan worden en de behandeling hierop aangepast. Terwijl bij patiënten positief voor *HLA-DRB1**0301 gekozen kan worden voor een meer afwachtend beleid. Daarnaast tonen onze studieresultaten dat een verhoogd percentage lymfocyten in de BAL prognostisch ongunstig is, terwijl een hoge CD4+/CD8+ ratio in de BAL prognostisch gunstig is.

In **hoofdstuk 5** onderzoeken wij een nieuwe eenheid, de 'total lung glycolysis' (TLuG) voor het kwantitatief uitdrukken van ziekteactiviteit op een ^{18}F-FDG PET/CT. In toenemende mate wordt gebruik gemaakt van ^{18}F-FDG PET/CT in sarcoïdose. ^{18}F-FDG PET/CT wordt ingezet voor verschillende doeleinden, zoals het in kaart brengen van ziekteactiviteit, het monitoren van ziekte en het bepalen van therapierespons. Tot op heden wordt in de meeste sarcoïdose onderzoeken naar ^{18}F-FDG PET/CT gebruik gemaakt van de maximum standardised uptake value (SUVmax) voor het kwantificeren van ziekteactiviteit. De SUVmax beschrijft de waarde van de pixel met de hoogste opname en dekt niet de totale ontstekingsactiviteit van de longen. De TLuG is de som van het metabole volume van de longen en de mean standardised uptake value, (SUVmean). In deze studie vergelijken wij de TLuG met de SUVmax in 27 patiënten met ernstige sarcoïdose en correleren we deze ^{18}F-FDG PET/CT markers met de longfunctie parameters tijdens behandeling met het biologische medicijn infliximab. Onze resultaten tonen een matige correlatie tussen de verandering van TLuG en de verandering van longfunctie tijdens behandeling met infliximab. Daarnaast vinden wij een vergelijkbare correlatie tussen de verandering van SUVmax en de verandering van de longfunctie tijdens infliximab. Hoog TLuG en SUVmax voor het starten van infliximab zijn beiden voorspellend voor het verbeteren van de longfunctie.

Concluderend zijn de TLuG en de SUVmax gelijkwaardig in het bepalen van long functionele respons op therapie en het voorspellen van respons op therapie. Omdat bij het bepalen van TLuG het aftekenen van de longen handmatig moet worden bijgesteld is dit een meer tijdrovende biomarker dan het bepalen van de SUVmax. Om deze reden raden wij aan de SUVmax te gebruiken als biomarker voor therapie respons en als prognostische ^{18}F-FDG PET/CT PET-marker.

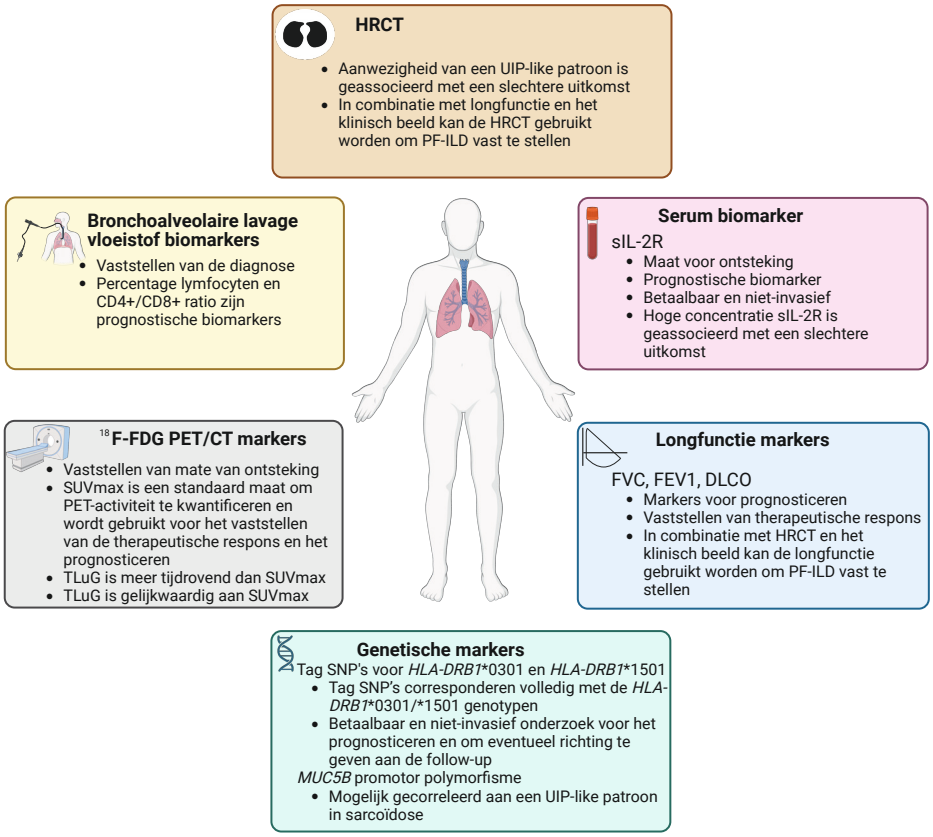
In **hoofdstuk 6** beschrijven wij de effectiviteit en de veiligheid van de biosimilar van infliximab, namelijk Inflectra®, in 29 patiënten met ernstige sarcoïdose. Infliximab wordt voorgeschreven als derdelijns behandeling in patiënten met therapieresistente sarcoïdose. Infliximab is een biological oftewel een biologisch medicijn bestaande uit natuurlijke eiwitten, dit maakt het productieproces kostbaar. Biosimilars zijn gelijkwaardig aan biologicals in effectiviteit en veiligheid, echter ze zijn minder kostbaar dan de biologicals. Het gebruik van biosimilars was binnen sarcoïdose nog niet onderzocht. Behandeling met Inflectra® gaf na zes maanden een significante verbetering van de longfunctie. Tevens zagen wij een afname van de ontstekingsactiviteit met een significante daling van de SUVmax en de sIL-2R na behandeling met Inflectra®. De gezondheidsgerelateerde kwaliteit van het leven nam na de behandeling met Inflectra® significant toe. Antistofvorming bij gebruik van biologische medicijnen kan ertoe leiden dat het medicijn ineffectief wordt. In geen van de patiënten uit ons cohort waren er aanwijzingen dat er antistoffen waren gevormd tegen Inflectra®. In vier patiënten traden ernstige bijwerkingen op leidend tot een ziekenhuisopname. Echter, na herstel van de ziekenhuisopname is Inflectra® in alle patiënten zonder problemen hervat. Samenvattend wijzen onze data erop dat Inflectra® vergelijkbaar is met de originele biological Remicade® in effectiviteit en veiligheid. Hopelijk verhoogt de introductie van de goedkopere biosimilars de toegankelijkheid wereldwijd tot deze effectieve derdelijns behandeling met biologische medicijnen. Biologische medicijnen worden volgens de nieuwste richtlijn van de European Respiratory Society (ERS) van 2021 aangeraden als derdelijns behandeling in patiënten met sarcoïdose in de longen, als patiënten persisterend ziekteactiviteit hebben ondanks tweedelijns behandeling met methotrexaat of azathioprine. Echter, tot op heden zijn er geen hoogkwalitatieve studies verricht naar de timing wanneer idealiter derdelijns behandeling geïnitieerd moet worden. Toekomstige onderzoeken zijn nodig om meer inzicht te geven in de farmacotherapeutische strategieën van sarcoïdose om de vigerende richtlijnen te verduidelijken.

In **hoofdstuk 7** beschrijven wij de karakteristieken van 106 patiënten met ernstige pulmonale sarcoïdose. Sarcoïdose patiënten met een diffusiecapaciteit van de longen van minder dan 50% van de voorspelde waarde werden geïncludeerd. Daarnaast biedt dit hoofdstuk meer inzicht in het progressieve fibroserende fenotype (PF-ILD) binnen sarcoïdose patiënten. In dit cohort met patiënten met ernstige sarcoïdose van de longen heeft het merendeel van de patiënten een stabiele of zelfs verbeterde longfunctie na twee jaar follow-up. De overall 5- en 10-jaars mortaliteit zijn respectievelijk 11% en 16%. Onafhankelijke voorspellers voor transplantatie-vrije mortaliteit zijn: pulmonale hypertensie, PF-ILD en een usual interstitial pneumonia-like (UIP-like) patroon op de CT-scan. Vijftien procent van de patiënten had PF-ILD en patiënten met dit fenotype

hadden een significant hogere mortaliteit van 43% versus 13% in de groep patiënten zonder PF-ILD. Samenvattend kunnen we concluderen dat de meeste patiënten met ernstige pulmonale sarcoïdose long functioneel stabiel blijven. Onze resultaten wijzen erop dat PF-ILD relatief zeldzaam is in sarcoïdose, echter dit fenotype gaat gepaard met een hoge mortaliteit. Tot voor kort waren fibroseremmers alleen voorbehouden voor patiënten met IPF. Recentelijk heeft de INBUILD-studie veelbelovende resultaten laten zien van de behandeling van longfibrose patiënten met een andere diagnose dan IPF welke voldoen aan de kenmerken van PF-ILD met fibrose-remmers. In het licht van de resultaten van de INBUILD-studie en onze studieresultaten kan overwogen worden sarcoïdose patiënten met PF-ILD te behandelen met fibrose-remmers. De huidige richtlijnen adviseren het besluit om te starten met fibroseremmers af te wegen van patiënt-tot-patiënt. In de huidige richtlijn wordt PF-ILD progressieve pulmonale fibrose (PPF) genoemd. De criteria voor het vaststellen van PPF verschillen van die van PF-ILD, namelijk de tijdsduur waarin progressie wordt vastgesteld is bij PPF één jaar versus twee jaar bij PF-ILD. De incidentie van PPF in sarcoïdose is tot op heden onbekend. Studies met grotere aantallen van sarcoïdose patiënten met PF-ILD zijn noodzakelijk om meer inzicht te bieden in deze behandeloptie voor patiënten met fibroserende sarcoïdose om de huidige richtlijnen verder aan te scherpen.

CONCLUSIES

Vanwege het heterogene karakter van sarcoïdose zijn gevalideerde biomarkers wenselijk voor het diagnosticeren, prognosticeren, voorspellen van het effect van therapie, monitoren van ziekteactiviteit of het vaststellen van een respons op therapie. De onderzoeken in deze thesis bieden nieuwe inzichten over bekende en nieuwe biomarkers voor sarcoïdose, waardoor deze beter geïmplementeerd kunnen worden in de dagelijkse klinische praktijk. In figuur 2 staan de biomarkers beschreven die zijn bestudeerd en op welke manier ze kunnen worden ingezet in de dagelijkse klinische praktijk. Tot op heden is de 'perfecte biomarker' binnen sarcoïdose niet gevonden. Het valideren en standaardiseren van een panel van meerdere biomarkers zou uitkomst kunnen bieden. Tevens biedt deze thesis nieuwe inzichten in de kleine groep PF-ILD patiënten binnen sarcoïdose, een ernstige vorm van pulmonale sarcoïdose. Het verwerven van kennis over de implementatie van biomarkers en fenotypes in sarcoïdose zal hopelijk in de toekomst bijdragen aan meer gepersonaliseerde zorg, waarbij de behandeling beter kan worden afgestemd op de individuele behoeften van de patiënt en controles bij een te verwachten gunstig beloop wellicht zelfs overgenomen zouden kunnen worden door de huisarts.



Figuur 2. Schematisch overzicht van de verschillende biomarkers die zijn bestudeerd in deze thesis en op welke manier ze kunnen worden gebruikt in de klinische praktijk.
HRCT= high-resolution computed tomography; UIP= usual interstitial pneumonia; PF-ILD= progressive fibrosing interstitial lung disease; sIL-2R= soluble interleukin 2 receptor; ^{18}F-FDG PET/CT= fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography; SUVmax= maximum standardised uptake value; TLuG= total lung glycolysis; FVC= forced viral capacity; FEV1= forced expiratory volume in one second; DLCO= diffusing capacity of the lungs for carbon monoxide; SNP= single nucleotide polymorphism; *MUC5B*= Mucin 5B.

LIST OF PUBLICATIONS

Schimmelpennink MC, Meek DB, Vorselaars ADM, Langezaal LCM, van Moorsel CHM, van der Vis JJ, Veltkamp M, Grutters JC. Characterization of the PF-ILD phenotype in patients with advanced pulmonary sarcoidosis. *Respir Res*. 2022;23(1):169.

Beijer ED, Roodenburg C, **Schimmelpennink MC**, Grutters JC, Meek B, Veltkamp M. Elevated Serum amyloid A levels are not specific for sarcoidosis but associate with a fibrotic pulmonary phenotype. *Cells*. 2021;10(3):585.

Schimmelpennink MC, Quanjel MJR, Vorselaars ADM, Wiertz IA, Veltkamp M, van Moorsel CHM, Grutters JC. Value of serum soluble interleukin-2 receptor as a diagnostic and predictive biomarker in sarcoidosis. *Expert Rev Respir Med*. 2020;14(7):749–756.

Schimmelpennink MC, Vorselaars ADM, Veltkamp M, Keijsers RGM. Quantification of pulmonary disease activity in sarcoidosis measured with 18F-FDG PET/CT: SUVmax versus total lung glycolysis. *EJNMMI Res*. 2019;9(1):54.

Karakaya B, **Schimmelpennink MC**, Kocourkova L, van der Vis JJ, Meek B, Grutters JC, Petrek M, van Moorsel CHM. Bronchoalveolar lavage characteristics correlate with HLA tag SNPs in patients with Löfgren's syndrome and other sarcoidosis. *Clin Exp Immunol*. 2019;196(2):249–258.

Schimmelpennink MC, Vorselaars ADM, Grutters JC; Biomarkers in sarcoidosis; In *Sarcoidosis: A Clinician's Guide*. 2018; 219-238.

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;138:S7–S13.

CONTRIBUTING AUTHORS

F.T. van Beek
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands

H.A. Crommelin
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Clinical Pharmacology, St. Antonius Hospital, Nieuwegein, The Netherlands

V.H.M. Deneer
Department of Clinical Pharmacology, Division of Laboratory and Pharmacy, University Medical Center Utrecht, The Netherlands

J.C. Grutters
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Pulmonology, Division of Heart and Lungs, University Medical Centre Utrecht, Utrecht, the Netherlands

B. Karakaya
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands

R.G.M. Keijsers
Department of Nuclear Medicine, St. Antonius Hospital, Nieuwegein, The Netherlands

L. Kocourkova
Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

L.C.M. Langezaal
Department of Radiology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands

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

D.B. Meek
Department of Radiology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Radiology, Treant Healthcare Group, Emmen, Hoogeveen, Stadskanaal, The Netherlands

C.H.M. van Moorsel
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Pulmonology, Division of Heart and Lungs, University Medical Centre Utrecht, Utrecht, the Netherlands

M. Petrek
Department of Pathological Physiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
University Hospital Olomouc, Olomouc, the Czech Republic

M.J.R. Quanjel
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands

M. Veltkamp
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Pulmonology, Division of Heart and Lungs, University Medical Centre Utrecht , Utrecht, The Netherlands

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

A.D.M. Vorselaars
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Pulmonology, Division of Heart and Lungs, University Medical Centre Utrecht, Utrecht, the Netherlands

I.A. Wiertz
Department of Pulmonology, Interstitial Lung Diseases Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands

CURRICULUM VITAE

Milou Schimmelpennink was born on December 26th, 1990, in Abcoude. She graduated from Hervormd Lyceum Zuid in Amsterdam in 2009. After graduating secondary school, she started medical school at the University of Groningen. During her medical study she worked as an intern at the University Medical Center of Groningen, Isala clinics in Zwolle and Meppel, and the St. Elisabeth hospital in Curaçao. In her final year, she organised a medical intern's conference in Zwolle. In 2017, she joined the Interstitial Lung Disease Center of Excellence as a researcher, initiating her PhD project under the supervision of Prof. dr. J.C. Grutters. During the PhD trajectory she was an active member of "Jong Antonius".

In 2019 she started as a resident (not in training), initially at the department for internal medicine at the St. Antonius hospital in Utrecht. Subsequently, she transitioned to the department anesthesiology and intensive care at the St. Antonius hospital in Utrecht and Nieuwegein. Finally, in preparation to her training as general practitioner she worked for several months at a high care unit of the psychiatry department at Altrecht in Utrecht. In 2021 she started as general practitioner in training at the University of Amsterdam. Currently, she resides in Amsterdam and is finishing her final year of her general practitioner training.

DANKWOORD

En dan is het nu tijd voor het dankwoord. Ik ben mij ervan bewust hoe bevoorrecht ik ben dat ik dit traject heb mogen afleggen. Het was een bijzondere werkervaring om mijn carrière mee te starten.

Ten eerste wil ik alle patiënten bedanken die hun gegevens ter beschikking hebben gesteld voor onderzoek, zonder hen was dit proefschrift niet tot stand gekomen.

Daarnaast wil ik de leden van de beoordelingscommissie bedanken voor het lezen en beoordelen van deze thesis.

Prof. dr. Grutters, beste Jan, allereerst wil ik jou bedanken voor de kans die mij is gegund om deel uit te mogen maken van het onderzoeksteam. Daarnaast wil ik je bedanken voor het kritisch lezen en verbeteren van mijn stukken en voor de scherpe input tijdens alle voortgangsgesprekken en vrijdagmiddag besprekingen.

Dr. Veltkamp, beste Marcel, om in jouw woorden te blijven is er dan eindelijk definitief "een klap op gegeven", het is af! Jouw enthousiasme voor sarcoïdose is niet te stuiten, daardoor kon ik na onze bijeenkomsten elke keer met een dosis nieuwe energie aan de slag. Dank voor alle keren dat ik bij jou terecht kon voor mijn onderzoeksvragen.

Dr. Vorselaars, beste Renske, ik heb mij tijdens dit traject vaak verwonderd hoe jij naast je drukke baan als longarts altijd op korte termijn (zelfs tijdens verlof en vakanties!) tijd wist vrij te maken voor mijn onderzoeksvragen en het lezen van mijn artikelen. Het is duidelijk dat je een grote passie voor onderzoek hebt. Ik wil je bedanken voor je enorme betrokkenheid en interesse van het begin tot het einde.

Dr. Van Moorsel, beste Coline, wat ontzettend fijn dat jouw deur voor iedere onderzoeker altijd open staat, voor zowel grote en kleine vragen. Maar wat ik nog veel meer kan waarderen is jouw enorme eerlijkheid en directheid, wat soms leidt tot hilariteit. Mailtjes van jou bevatten nooit meer dan enkele kernachtige woorden. Deze efficiëntie vind ik prachtig.

Dr. Keijsers, beste Ruth, bedankt voor jouw uitleg en het beoordelen van de PET-scans. Ook bedankt voor de geduldige uitleg over hoe de 'total lung glycolysis' moest worden beoordeeld, zodat ik er zelf mee aan de slag kon. Ook wil ik jou bedanken voor het kritisch lezen van mijn stukken.

Beste radiologen, Drs. Meek en Drs. Langezaal, bedankt voor jullie inzet en harde werken. Onder grote tijdsdruk hebben jullie toch een heleboel HRCT's kunnen beoordelen en hierdoor heb ik het laatste artikel dan eindelijk kunnen publiceren.

Dr. Korenromp, beste Ingrid, dank voor jouw flexibele en fijne begeleiding bij de laatste loodjes en voor de wijze raad (zoals op tijd pauze nemen). Doordat jij mij hebt geholpen met het structureren van de laatste stukken heb ik dit proefschrift kunnen afronden. Je hebt me enorm geholpen en daar ben ik je heel dankbaar voor.

Speciale dank aan Annette, niet alleen heb je labwerk verricht en meegeschreven aan meerdere artikelen, ook wist je, ondanks je drukke agenda, altijd tijd vrij te maken om mijn planning en onderzoek beslommeringen aan te horen. Als doorgewinterde paranimf kon ik je altijd vragen om een paar goede adviezen.

Beste Bekir, bij het Löfgren artikel begon mijn ILD-avontuur natuurlijk! Bedankt voor het wegwijs maken in de sarcoïdose-wereld.

Daarnaast wil ik alle andere co-auteurs bedanken die kritisch hebben meegelezen en meegeschreven aan de artikelen.

Lieve ILD collega's, Lieve Aernoud, Els, Sofia, Mark, Ivo, Marjolijn, Annelies, Michelle, Inge, Thijs, Bekir, Karin, Dymph, Nynke, Yvonne, Helmi, Karlijn, Lisette, Montse, Raisa en Kim, dank voor alle gezellige momenten op de onderzoekskamer. Aernoud, bedankt dat ik bij je terecht kon voor de laatste promotie tips! De potten koffie waren vaak niet aan te slepen en deze momenten gingen vaak gepaard met een gezellig verhaal of een paar praktische tips. Ik heb genoten van alle ILD-uitjes, borrels, pubquizzen, festivals en natuurlijk de congressen. Dankzij jullie was het promoveren een feestje!

Mijn huisartsen-collega's wil ik bedanken voor de belangstelling en het begrip tijdens de afgelopen jaren. Ik vind het heerlijk om huisarts in opleiding te zijn en ik ben blij dat ik een vak heb gevonden waar ik zó enthousiast van word. Ik kan niet wachten om straks nog meer energie in dit prachtige vak te kunnen steken.

Vrienden, Lieve Rupsjes Nooitgenoeg, ABC-homies, Prootógennèten, wintersport-/tennis-maatjes en alle andere vrienden, wat heerlijk dat ik omringd ben door zulke lieve, warme en kleurrijke vrienden. Bedankt voor alle gezelligheid, vriendschap en mooie avonturen. Laten we proosten op nog vele feestjes de komende jaren!

Lieve schoonfamilie, lieve Marijke, Franz, Max & Cheryl, dank voor jullie belangstelling (en medeleven) en natuurlijk de flexibiliteit in extra oppasdagen voor Hannah.

Lieve familie, lieve mama, Jiska & Arno, Gwenny & Eduard, bedankt voor jullie interesse in mijn onderzoek, het begrip als ik weer verstek moest laten gaan en jullie bemoedigende woorden. Helaas heeft papa dit niet meer mogen meemaken, hij zou dit vast een hele feestelijke dag hebben gevonden. De kleuring van een granuloom is paars, papa's lievelingskleur, hoe toepasselijk. Met de uitbreiding met al het kleine gespuis (kleine Elias, Lucy, Céline, Hannah en Sarah) zijn de zondagen nu helemaal omgetoverd tot een gezellige bende.

Lieve paranimfen, lieve Jiska & Carolien, ik ben dankbaar dat jullie deze dag aan mijn zijde willen flaneren. Jis, toen ik jou vroeg om mijn paranimf te zijn moest je eerst even opzoeken wat dit betekent, maar daarna was je direct enthousiast. Ik voel me gesterkt door jou, dat jij als grote zus, deze dag mijn paranimf wilt zijn. Lieve Caro, amice uit 't Hoge Noorden, hoe fijn dat je op deze dag wilt souffleren, binnenkort pilsen we 't af in de Poele. Helaas wonen we mijlenver bij elkaar vandaan, maar als we bij elkaar zijn is het altijd weer als vanouds.

Lieve Peet, lieve Hansje, mijn twee lievelingsmensen, thuiskomen bij jullie in onze gezellige chaos is elke dag opnieuw een feestje. Ik ben gezegend twee van zulke lieve vrolijkerds naast me te mogen hebben!