The Many Faces of Sarcoidosis

Novel insights into pathogenesis, diagnostics and therapy

Lieke Susan Josephien Kamphuis

Printing this thesis was supported by: Sarcoïdose Belangenvereniging Nederland, AbbVie B.V. and ILD-care foundation.

www.ildcare.nl

The Many Faces of Sarcoidosis

Novel insights into pathogenesis, diagnostics and therapy

De vele gezichten van sarcoïdose

Nieuwe inzichten in pathogenese, diagnostiek en behandeling

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam

op gezag van de
rector magnificus

Prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 25 mei 2016 om 13.30 uur.

door

Lieke Susan Josephien Kamphuis
Geboren te Oldenzaal

Erasmus University Rotterdam

Promotiecommissie

Promotor:	Prof.dr. P.M. van Hagen
Copromotoren:	Dr. J.A.M. van Laar Dr. M.C. van Zelm
Overige leden:	Prof.dr. R.W. Hendriks Prof.dr. J.C. Grutters Prof.dr. L.J. Hofland

Contents

Chapter 1

General introduction

Parts of this introduction are based on:
Sarcoidosis: changing insights in therapy

Kamphuis LS, van Laar JA, Kuijpers RW, Missotten T, Thio HB, van Hagen PM

Ned Tijdschr Geneeskd. 2010;154:A1685

Introduction

Sarcoidosis is a granulomatous disorder of unknown cause, affecting multiple organs. It was first described in 1877 as a skin disease by a dermatologist, Jonathan Hutchinson (1). However, the most famous synonym worldwide for sarcoidosis is Besnier-Boeck-Schaumann disease (2).

Mostly sarcoidosis develops before the age of 50 years, with an incidence peaking at 20 to 39 years (3). The worldwide prevalence varies from 2 to 80 per 100,000 (4). In the Netherlands the prevalence is estimated to be 50 per 100,000 (5). However, in 30-60% of the cases the prevalence may be underestimated by the asymptomatic signs of the disease (5). In Afro-Americans the incidence is three times higher compared to Caucasians and it is also more likely to be chronic and fatal in Afro-Americans (3,6).

Seasonal clustering of sarcoidosis has been reported between March and July and occupation clustering was recorded in health care workers, naval aircraft servicemen and fire fighters (7).

Immunopathogenesis and genetics

It is presumed that granulomas play a central role in the pathogenesis of sarcoidosis. In normal conditions granulomas are produced to lock up pathogens leading to a protection of the surrounded tissue against an inflammatory reaction. The hypothesis in sarcoidosis is that granulomas are formed by an interaction between antigen presenting cells (APC) and activated T-lymphocytes, mainly CD4+ T cells. This leads to a release of cytokines, (interleukin(IL)-2 and interferon (IFN)-γ), who are triggering macrophages to produce tumor necrosis factor-α (TNF-α) and strengthen the local inflammation response by releasing a cascade of several cytokines (Figure 1) (8).

The clustering written above support the idea that an agent might be the trigger to induce sarcoidosis. However, no agent has been identified as the cause of sarcoidosis, so far.

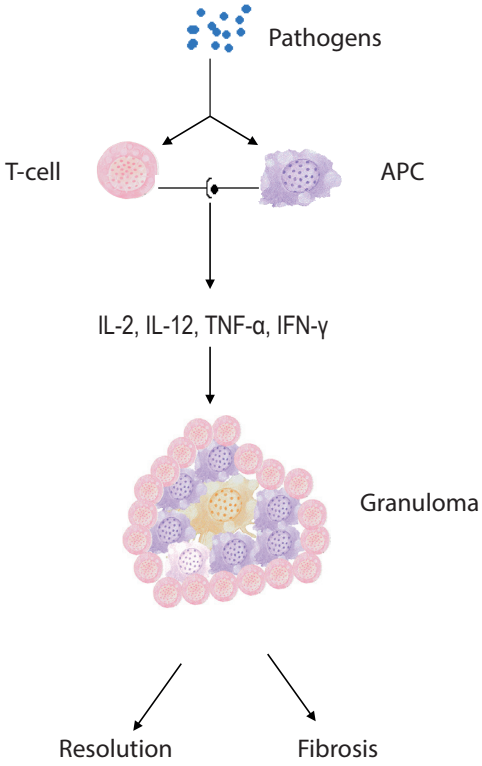
In the 1990's large numbers of B-lymphocytes were found in intergranulomatous areas of lymph nodes in sarcoidosis patients (9). However, until now the role of the B-lymphocyte in sarcoidosis remains open. B-lymphocytes are responsible for the humoral response. They contribute to the immune system by production of antigen-specific antibodies.

TNF-α stimulates the differentiation of monocytes into macrophages and the differentiation of macrophages into epithelioid cells, where after giant

cells are produced. Those cells are generated because of the junction of epithelioid cells (10). Treated sarcoidosis patients show a spontaneous high release of TNF-α in alveolar macrophages compared to a control group (11). That is why TNF-α seems to play a central role in the pathogenesis of sarcoidosis. Another argument that TNF-α plays a major role are the recent results of genetic analysis in sarcoidosis patients. De presence of TNF-α-G308A-allel in HLA-DRB1*03 positive patients is predictive for a better prognosis in pulmonary sarcoidosis (12). Furthermore, there is a connection between TNF-α-G308A and HLA-DRB1*03 expression in patients with acute sarcoidosis i.e. Löfgren's syndrome. HLA-DRB1*03 positive patients with this syndrome have a higher chance to recover than HLA-DRB1*03 negative patients (13).

Mutations which can lead to sarcoidosis are found in children younger than four years and is called "early onset sarcoidosis (EOS)" and the "Blau syndrome" (14). These rare autosomal dominant disorders with arthritis and granulomatous inflammation of the eyes and skin is caused by mutations in the nucleotide binding oligomerisationdomain (NOD)-2 gene. This leads to a hyperreactive defense mechanism with granuloma production in which TNF-α and IFN-γ seem to play a major role (14, 15).

Figure 1. The hypothesized immunopathogenesis in sarcoidosis.



Clinical manifestation

The clinical presentation of sarcoidosis depends on which organ is affected. Given the fact that any organ can be involved, the presentation varies widely in symptoms and clinical findings (16). Nonspecific symptoms like fatigue, weight loss and fever may be indicative for active sarcoidosis. Acute presentation, i.e., Löfgren's syndrome, presents with acute arthritis, erythema nodosum and bilateral hilar lymphadenopathy. Heerfordt's syndrome is another rare syndrome of sarcoidosis and presents with a triad of facial nerve palsy, uveitis and parotid gland enlargement (17).

Pulmonary involvement

The lung is involved in the majority of patients and symptoms like dyspnea and cough usually implies active disease. Depending on the severity of the inflammation in the lung, pulmonary function tests may reveal restricted lung volumes and/or impaired diffusion capacity (18).

Skin involvement

Cutaneous manifestations occur in about 25% of sarcoidosis patients (19). Patients presenting with only sarcoid like lesions on the skin will mostly be found to develop other evidence of systemic sarcoidosis (20). Common signs of skin involvement are maculopapular lesions, lupus pernio, cutaneous or subcutaneous nodules and infiltrative scars (19, 20). Cutaneous involvement is more common in patients of African descent than in white (21). Lupus pernio seems to be associated with chronic sarcoidosis and a worse prognosis (23). The most common nonspecific lesion is erythema nodosum. This occurs in 3% to 34% of patients with systemic sarcoidosis (19). In contrast to lupus pernio, patients with erythema nodosum tend to have a good prognosis and not go on to chronic disease (19).

Ocular involvement

The frequency of ocular involvement differs between 10% to 50%, and includes extraocular disorders such as lacrimal gland swelling or sicca syndrome (23). Intraocular involvement seen as uveitis is a significant concern because it is likely to reduce the vision and to deteriorate the quality of life. All patients suspected for sarcoidosis should be referred to an ophthalmologist.

Neurological manifestations

Neurosarcoidosis, which is thought to occur in less than 10% of the patients, has a predilection for the base of the brain. However, any part of the central or peripheral nervous system may be involved (24, 25). The diagnosis of neurosarcoidosis is often very difficult, especially in patients who lack either pulmonary or systemic manifestations of sarcoidosis. About 50% of patients with nervous system sarcoidosis have facial nerve palsy (26). Peripheral nervous system involvement may cause muscle weakness, atrophy, sensory disturbance or deep tendon-reflex loss in the distribution of the affected nerve (27).

Cardiac involvement

Cardiac involvement is the leading cause of death in sarcoidosis. An electrocardiography should therefore be performed at diagnosis (24). In a recent report

the incidence of cardiac sarcoidosis was 2.3% (6). The most frequent cardiac manifestations are arrhythmias, sudden death and congestive heart failure, but uncommon manifestations have also been reported.

Hypercalcemia

Hypercalcemia is a known feature in sarcoidosis with a prevalence of 5 to 11% (28, 29). Activated macrophages in sarcoid granulomas are capable to upregulate extrarenal 1α-hydroxylase, the enzyme which converts 25-hydroxy vitamin D (25-(OH)D) to its active form, 1,25-dihydroxy vitamin D (1,25(OH)2D). This can result in hypercalcemia (30). Other mechanisms for hypercalcemia in sarcoidosis are expression of parathyroid hormone-related protein (PTH-rP) in sarcoid macrophages what may exert an autocrine action of 1α-hydroxylase activity and increased levels of serum IFN-γ (31-34). IFN-γ stimulates the production of 1,25(OH)2D by alveolar macrophages (35).

Diagnostic procedures

Sarcoidosis is a diagnosis of exclusion. There exist neither a pathognomonic clinical feature nor a perfect diagnostic test. Missed diagnosis and overdiagnosis are therefore common. Currently the diagnosis of sarcoidosis is based on the criteria as proposed by the American Thoracic Society, European Respiratory Society and World Association of Sarcoidosis and Other Granulomatous Disorders (36). This means histological confirmation of non-caseating granulomas, clinical signs of activity and radiological pictures are compatible with sarcoidosis.

Among serum markers in sarcoidosis, serum angiotensin converting enzyme (ACE) is the most used (36). It is produced by epitheloid cells and alveolar macrophages and serum levels of ACE are thought to reflect the extent of granulomatous inflammation (36). However, elevated levels of serum ACE can be found in other medical conditions as well. Its sensitivity is low (55%), but when the diagnosis sarcoidosis is confirmed the specificity is 99% (37). Therefore it is suggested to be useful for treatment monitoring (38, 39).

Elevated levels of soluble IL-2 receptor (sIL-2R) in serum have been shown to be associated with active disease (40). IL-2 receptors are found on the surface of T- and B-lymphocytes, monocytes and macrophages (41-43). Its soluble form is associated with cellular immune reactions and might

therefore be increased in sarcoidosis. The bronchoalveolar lavage in sarcoidosis patients reveals an increased number of T-lymphocytes expressed to an increased CD4+/CD8+ ratio (44). Despite their diagnostic value, the number of lymphocytes and the CD4+/CD8+ ratio do not have a predictive value.

Visualization techniques in sarcoidosis are most common a chest X-ray and computed tomography (CT) – scan. Other used techniques are the Fluorine-18 fluorodeoxyglucose positron emission tomography (18F-FDG PET) and the somatostatin receptor scintigraphy (SRS).

Treatment

Treatment of symptoms is essential in sarcoidosis. Systemic treatment is often started when there are signs of organ damage or invalidating clinical signs. The first step of symptom treatment are local corticosteroids, NSAID's or other pain medication. Hydroxychloroquine, an anti malaria drug with immunomodulatory effects, may prevent for organ damage (4). Treatment with corticosteroids is indicated by severe general signs, vital organ damage or hypercalcemia (4, 6, 11). In case of insufficient therapeutic efficacy of side effects other immunosuppressives could be used, see Table 1.

Corticosteroids have an extensive immunosuppressive effect under which the inhibition of T-lymphocytes TNF-α. There are several conventional immunosuppressives with a comparable mechanism. For example pentoxyifylline is suppressing the release of TNF-α by alveolair macrophages. However, the used dosage of pentoxifylline was causing a lot of gastrointestinal side effects (45).

If a patient has sarcoidosis for more than two years, it is called chronic sarcoidosis (5). A dosage of prednisolon under 7.5 mg/day in chronic sarcoidosis can be continued. In cases of higher dosages it is indicated to switch to another immunosuppresive, like methotrexate, azathioprine, cyclophosphamide or mycofenolate mophetil. The next step in the treatment of chronic sarcoidosis are TNF-α blockers (11). The development of TNF-α blockers was the beginning of a new chapter in the treatment of sarcoidosis. TNF-α is probably the most important cytokine in sarcoidosis and inhibition of this cytokine has besides in sarcoidosis also in a lot of other systemic diseases a positive effect (4).

Currently there are four TNF-α blockers available: infliximab, adalimumab, etanercept and golimumab. Infliximab and adalimumab propably induce apoptosis of TNF-α binding cells, what contributes to the inhibition of granuloma formation. Infliximab is the most investigated of this four and has shown to be effective in other inflammatory diseases (46). Recently, the TNF-α blocker infliximab demonstrated significant clinical benefit in large cohorts of patients with pulmonary manifestations of sarcoidosis (47, 48). In two double-blinded randomized, and placebo controlled trials of patients with pulmonary sarcoidosis a favorable clinical response was demonstrated (49, 50). Etanercept has been reported effective in case reports, but the only trial so far resulted in detoriation of almost a third of the patients with acute pulmonary sarcoidosis (51-53). Adalimumab could be an important additional option in the treatment with biologicals.

Other biologicals available for the treatment of sarcoidosis are rare. There are a few cases of a positive effect of anti B-lymphocyte therapy, i.e. rituximab (anti-CD20).

Table 1. Current immunosuppressive treatment in sarcoidosis.

Medication	**Dose**
Glucocorticosteriods	
Prednisone	start 5-40 mg/day
	maintenance <10 mg/day
Anti malaria drugs	
Hydroxychloroquine	200-400 mg/day
Cytotoxic medication	
Azathioprine	50-150 mg/day
Cyclophosphamide	50-150 mg/day oral
	500-1500 mg Q2-4W i.v.
Methotrexate	10-25 mg/weekly
Sodium mycophenolate/mycophenolate mofetil	720 mg/1000 mg twice daily
Cytokine modulating medication	
Infliximab	50-10 mg/kg Q4-8W
Adalimumab	40-80 mg Q1-2W
Thalidomide	50-100 mg/day

Prognosis

The prognosis of acute sarcoidosis (Löfgren's syndrome) is good and spontaneous resolution frequently occurs within two years. The mortality rate of sarcoidosis is approximately 5% (54). In more than 50% of the patients a spontaneous remission is seen within three years (4).

Sarcoidosis can be classified in five stages according to the Scadding system with chest X-rays (55, 56). Stage 0, normal appearance; stage I, bilateral hilar lymphadenopathy alone; stage II, bilateral hilar lymphadenopathy and parenchymal shadowing; stage III, parenchymal shadowing alone; stage IV, fibrosis. The remission rate is related to the radiographic stage, but may vary due to the different ethnic backgrounds (3, 57-60). Approximately 45-80% of the patients with stage I will recover from sarcoidosis, which is 30-70% in stage II, 10-20% in stage III and 0% in stage IV.

Aims of this thesis

Sarcoidosis is a granulomatous multisystem disease of unknown origine what makes it difficult to manage because of a lack of disease activity criteria, specific sarcoid biomarkers, and limited therapeutic possibilities. In this thesis we have focused on B cells in the pathophysiology of sarcoid granulomas in order to detect new disease targets, imaging and biomarkers in sarcoidosis, and new therapeutic approaches in sarcoidosis.

References

1. Sharma OP, Shigemitsu H. A historical sketch; life and time of Jonathan Hutchinson (1828-1913), the first sarcoidologist. Sarcoidosis Vasc Diffuse Lung Dis. 2008;25:71-75.
2. Sarcoidosis—the beginning: historical highlights of personalities and their accomplishments during the early years. J Natl Med Assoc. 1984;76:887-896.
3. Rybicki BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzi MC. Racial differences in sarcoidosis incidence: a 5-year study in a health maintenance organization. Am J Epidemiol. 1997;145:234-241.
4. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
5. Hillerdal G, Nou E, Osterman K. Sarcoidosis: epidemiology and prognosis. A 15-year European study. Am Rev Respir Dis. 1984;130:29-32.
6. Baughman RP, Teirstein AS, Judson MA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001;164:1885-1889.
7. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361:1111-1118.
8. Lazarus A. Sarcoidosis: epidemiology, etiology, pathogenesis, and genetics. Dis Mon. 2009;55:649-660.
9. Fazel SB, Howie SEM, Krajewski AS, Lamb D. B lymphocyte accumulations in human pulmonary sarcoidosis. Thorax. 1992;47:964-967.
10. Zissel G, Prasse A, Müller-Quernheim J. Sarcoidosis – immunopathogenetic concepts. Semin Respir Crit Care Med. 2007;28:3-14.
11. Baughman RP, Lower EE, Drent M. Inhibitors of tumor necrosis factor (TNF) in sarcoidosis: who, what, and how to use them. Sarcoidosis Vasc Diffuse Lung Dis. 2008;25:76-89.
12. Wijnen PA, Nelemans J, Verschakelen JA, Bekers O, Voorter CE, Drent M. The role of tumor necrosis factor alpha G-308A polymorphisms in the course of pulmonary sarcoidosis. Tissue Antigens. 2010;75:262-268.
13. Grunewald J, Eklund A. Löfgren's Syndrome. Am J Respir Crit Care Med. 2009;179:307-312.
14. Okafuji I, Nishikomori R, Kanazawa N, et al. Role of the NOD2 Genotype in the Clinical Phenotype of Blau Syndrome and Early-Onset Sarcoidosis. Arthritis Rheum. 2009;60:242-250.
15. Le Bourhis L, Benko S, Girardin SE. Nod1 and Nod2 in innate immunity and human inflammatory disorders. Biochem Soc Trans. 2007;35:1479-1484.
16. Judson MA, Baughman RP, Teirstein AS et al. Defining organ involvement in sarcoidosis: the ACCESS proposed instrument. ACCESS Research Group. A Case Control Etiologic Study of Sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 1999;16:75-86.
17. Heerfordt CF. Uber eine "Febris uveo-parotidea subchronica", an der Glandula parotis und der Uvea des Auges lokalisiert und hauftig mit Paresen cerebrospinaler Nerven kompliziert. Arch Opthalmol. 1909;70:254-273.
18. Müller-Quernheim J. The cytokine network in sarcoidosis. Eur Cytokine Netw. 1996;7:13-26.

19. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, Eklund A, Kitaichi M, Lynch J, Rizzato G, Rose C, Selroos O, Semenzato G, Sharma OP. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis. 1999;16:149-173.
20. James DG. Dermatological aspects of sarcoidosis. Quart J Med. 1959;28:109-124.
21. English JC, Patel PJ, Greer KE. Sarcoidosis. J Am Acad Dermatol. 2001;44:725-743.
22. Mana J, Marcoval J, Graelis J, Salazar A, Pyri J, Pujol R. Cutaneous involvement in sarcoidosis: relationship to systemic disease. Arch Dermatol. 1997;33:882-888.
23. Crick PP, Hoyle C, Smellie H. The eye in sarcoidosis. Br J Ophthalmol. 1961;45:461-481.
24. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med. 1997;336:1224-1234.
25. Sharma OP. Neurosarcoidosis. A personal perspective based on the study of 37 patients. Chest. 1997;112:220-228.
26. Zajicek JP, Scolding NJ, Foster O, Rovaris M, Evanson J, Moseley IF, Scadding JW, Thompson EJ, Chamoun V, Miller DH, et al. Central nervous system sarcoidosis-diagnosis and management. Q J Med. 1999;92:103-117.
27. Said G, Lacroix C, Plante-Bordeneuve V, Le Page L, Pico F, Presles O, Senant J, Remy P, Rondepierre P, Mallecourt J. Nerve granulomas and vasculitis in sarcoid peripheral neuropathy: a clinicopathological study of 11 patients. Brain. 2002;125:264-275.
28. Costabel U, Teschler H. Biochemical changes in sarcoidosis. Clin Chest Med. 1997;18:827-842.
29. Kamphuis LS, Bonte-Mineur F, van Laar JA, van Hagen PM, van Daele PL. Calcium and vitamin D in sarcoidosis: is supplementation safe? J Bone Miner Res. 2014;29:2498-2503.
30. Adams JS, Singer FR, Gacad MA, Sharma OP, Hayes MJ, Vouros P, et al. Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar macrophages in sarcoidosis. J Clin Endocrinol Metab. 1985;60:960-966.
31. Reichel H, Koeffler HP, Barbers R, Norman AW. Regulation of 1,25-dihydroxyvitamin D3 production by cultured alveolar macrophages from normal human donors and from patients with pulmonary sarcoidosis. J Clin Endocrinol Metab. 1987;65:1201-1209.
32. Falk S, Kratzsch J, Paschke R, Koch CA. Hypercalcemia as a result of sarcoidosis with normal serum concentrations of vitamin D. Med Sci Monit. 2007;13:CS133-136.
33. Inui N, Murayama A, Sasaki S, Suda T, Chida K, Kato S, et al. Correlation between 25-hydroxyvitamin D3 1 alpha-hydroxylase gene expression in alveolar macrophages and the activity of sarcoidosis. Am J Med. 2001;110:687-693.
34. Zeimer HJ, Greenaway TM, Slavin J, Hards DK, Zhou H, Doery JC, et al. Parathyroid-hormone-related protein in sarcoidosis. Am J Pathol. 1998;152:17-21.
35. Koeffler HP, Reichel H, Bishop JE, Norman AW. Gamma-Interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. Biochem Biophys Res Commun. 1985;127:596-603.
36. Costabel U, de Bois RM, Eklund A et al. Consensus conference: activity of sarcoidosis. Eur Respir J. 1994;7:624-627.
37. Sharma OP, Alam S. Diagnosis, pathogenesis, and treatment of sarcoidosis. Curr Opin Pulm Med. 1995;1:392-400.

38. Rust M, Bergmann L, Kuhn T et al. Prognostic value of chest radiograph, serumangiotensin-converting enzyme and T helper cell count in blood and in bronchoalveolar lavage of patients with pulmonary sarcoidosis. Respiration. 1985;48:231-236.
39. Hollinger WM, Staton GW, Jr, Fajman WA et al. Prediction of therapeutic response in steroid-treated pulmonary sarcoidosis. Evaluation of clinical parameters, bronchoalveolar lavage, gallium-67 lung scanning, and serum Angiotensin-converting enzyme levels. Am Rev Respir Dis. 1985;132:65-69.
40. Bargali E, Bianchi N, Margollicci M, Olivieri C, Luddi A, Coviello G, Grosso S, Rottolli P. Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. Scand J Clin Lan Invest. 2008;68:479-483.
41. Ina Y, Takada K, Sato T et al. Soluble interleukin 2 receptors in patients with sarcoidosis. Possible origin. Chest. 1992;102:1128-1133.
42. Malkovsky M, Loveland B, North M et al. Recombinant interleukin-2 directly augments the cytotoxicity of human monocytes. Nature. 1987;325:262-265.
43. Tsudo M, Uchiyama T, Uchino H. Expression of Tac antigen on activated normal human B cells. J Exp Med. 1984;160:612-617.
44. Yeager H, Jr., Williams MC, Beekman JF et al. Sarcoidosis: analysis of cells by bronchial lavage. Am Rev Respir Dis. 1977;116:951-954.
45. Zabel P, Entzian P, Dalhoff K, Schlaak M. Pentoxifylline in treatment of sarcoidosis. Am J Respir Crit Care Med. 1997;155:1665-1669.
46. Lochs H, Adler G, Beglinger C, Duchmann R, Emmrich J, Ewe K, Gangl A, Gasche C, Hahn E, Hoffmann P, et al. Anti-TNF antibody in Crohn's disease: status of information, comments and recommendations of an international working group. Z Gastroenterol. 1999;37:509-512.
47. Baughman RP, Costabel U, du Bois RM. Treatment of sarcoidosis. Clin Chest Med. 2008;29:533-548.
48. Baughman RP. Tumor necrosis factor inhibition in treating sarcoidosis: the American experience. Revista Portuguesa de Pneumonologia. 2007;13:47-50.
49. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Müller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES; Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med. 2006;174:795-802.
50. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller W Jr, Sands BE. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2006;23:201-208.
51. Khanna D, Liebling MR, Louie JS. Etanercept ameliorates sarcoidosis arthritis and skin disease. J Rheumatol. 2003;30:1864-1867.
52. Tuchinda C, Wong HK. Etanercept for chronic progressive cutaneous sarcoidosis. J Drugs Dermatol. 2006;5:538-540.
53. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. Chest. 2003;124:177-185.
54. Reich J. Mortality of intrathoracic sarcoidosis in referral vs population-based settings: influence of stage, ethnicity, and corticosteroid therapy. Chest. 2002;121:32-39.

55. Baughman RP, Teirstein AS, Judson MA, et al; Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001;164:1885-1889.
56. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. Br Med J. 1961;2:1165-1172.
57. Lynch JP, III, Ma YL, Koss MN et al. Pulmonary sarcoidosis. Semin Respir Crit Care Med. 2007;28:53-74.
58. Neville E, Walker AN, James DG. Prognostic factors predicting the outcome of sarcoidosis: an analysis of 818 patients. Q J Med. 1983;52:525-533.
59. Pietinalho A, Ohmichi M, Lofroos AB et al. The prognosis of pulmonary sarcoidosis in Finland and Hokkaido, Japan. A comparative five-year study of biopsy-proven cases. Sarcoidosis Vasc Diffuse Lung Dis. 2000;17:158-166.
60. Siltzbach LE. Sarcoidosis: clinical features and management. Med Clin North Am. 1967;51:483-502.

Chapter 2

Pathogenesis

2.1

Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis?

Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma GS, Dik WA, Thio HB, van Daele PL, van Velthoven ME, Batstra MR, van Hagen PM, van Laar JA

Am J Respir Crit Care Med. 2013;187:406-416

Abstract

Rationale

Recent observations of abnormal immunoglobulin responses and case reports describing successful B-cell ablabtive therapy suggest involvement of B cells in the pathogenesis of sarcoidosis.

Objectives

To investigate how abnormal B-cell maturation and function in patients with sarcoidosis contribute to disease.

Methods

Patients with sarcoidosis (n = 32) were included for detailed analysis by immunohistochemistry of tissue, flow cytometry of blood B-cell subsets, and serum immunoglobulin levels. Vaccination responses in patients with sarcoidosis to influenza virus and encapsulated bacteria and molecular analysis of immunoglobulin responses.

Measurements and main results

Perigranuloma localization of IgA-producing plasma cells and numerous B cells were found in affected tissues. Total blood B-cell numbers were normal, CD27+ memory B cells were significantly reduced, and CD27-IgA+ B cells were significantly increased; the results are normalized in patients treated with TNF-α blockers. Despite this, patients had normal serum immunoglobulin levels and normal antigen-specific immunoglobulin responses. IgA and IgG transcripts, however, showed high frequencies of somatic hypermutations and increased usage of ownstream IgG subclasses, suggestive for prolonged or repetitive responses.

Conclusions

The large B-cell infiltrates in granulomatous tissue and increased molecular signs of antibody maturation are indicative of direct involvement of B cells in local inflammatory processes in patient with sarcoidosis. Moreover, CD27-IgA+ B cells could be a marker for treatment with TNF-α blockers. These findings of B cells as ermerging key players provide a rationale for a systemic study on B-cell ablative therapy in patients with sarcoidosis.

Introduction

B cells are emerging as key players in several systemic autoimmune diseases that were generally considered T cell mediated, such as rheumatoid arthritis and granulomatosis with polyangiitis (1, 2). Sarcoidosis is a multisystem disorder of unknown etiology and is also considered T cell driven (3-5). The hallmark of sarcoidosis is the occurrence of non-caseating granulomas characterized by epithelioid- and CD4+ T cells surrounded by fibroblasts and CD8+ T cells accompanied by high tumor necrosis factor (TNF)-α levels (6, 7). In more advanced stages, a restricted repertoire of T cell receptors and oligoclonal T cell expansions suggest selective immunological activation (3).

Despite proven involvement of cellular immunity, several observations suggest abnormal B cell responses in sarcoidosis. These concern circulating immune complexes, hypergammaglobulinemia, autoantibody production, anergy and reduced frequencies of circulating CD27+ memory B cells (8-12). Moreover, treatment with B cell ablative therapy with the chimeric anti-CD20 antibody rituximab yields clinical improvement of sarcoidosis patients (13-16). Rituximab targets CD20, which is expressed on the surface of naive and memory B cells, but not on plasma cells (1). This suggests that naive and/or memory B cells play a pathophysiological role in sarcoidosis.

B cell involvement has been reported in other granulomatous diseases. In granulomatosis with polyangiitis, B cells are present in pathological lesions and B cell ablative therapy is highly effective (1). Furthermore, reduced numbers of blood memory B cells are observed in a granulomatous subgroup of patients with hypogammaglobulinemia (common variable immunodeficiency; CVID) (17-19). Recent findings reveal that in tuberculosis, a classical infectious granulomatous disease, B cells are present and participate in the development of tuberculous granulomas (20, 21).

After maturation from hematopoietic stem cells and generation of a functional immunoglobulin, B cells migrate from bone marrow to blood and peripheral lymphoid organs (22). B cells that specifically recognize antigen in lymphoid tissue with their specific surface immunoglobulin mature into immunoglobulin-secreting plasma cells and memory B cells (23). So, recirculating memory B cells in blood can reflect local responses (23). Detailed flow cytometric and molecular analyses of 6 blood memory B cell subsets and their origins from T cell dependent and T cell independent responses are recently described (24).

In this study, we phenotyped B cells in granulomas and blood of patients with sarcoidosis and studied their molecular functional characteristics and immunoglobulin responses to T cell-dependent and T cell-independent antigens. Although immunoglobulin responses appear normal, the presence of B cells in granulomas and altered memory B cell formation suggest pathophysiological involvement of B cells in sarcoidosis. Some of the results of these studies have been previously reported in the form of posters (25-27).

Material and methods

Patients

Diagnostic work-up, detailed studies of blood, tissue biopsies and vaccinations of 32 patients with sarcoidosis (Table 1) and control subjects were performed with informed consent according to the Declaration of Helsinki and guidelines of the Medical Ethics Committee of Erasmus MC.

Immunohistochemistry of tissue biopsies

Lymphocytes were studied in biopsies containing granulomas of 17 therapy-naive patients with sarcoidosis from various affected tissues, including lymph nodes of inguinal, axillary, and neck glands; skin; liver; heart; and bone marrow. Tissue slides were stained with hematoxylin and eosin and antibodies recognizing CD4 (SD35; Ventana, Tucson, Arizona), CD3 (UCHT1), CD8 (C8/144B), CD20 (L26), CD79a (JCB117) and IgG (A57H), IgA (polyclonal rabbit antibody; all from Dako Cytomation, Glostrup, Denmark), CD5 (4C7; Novocastra, Newcastle, UK), PAX5 (rabbit polyclonal antibody; Thermo Scientific, Waltham, MA), CD138 (B-A38; IQ Products, Groningen, The Netherlands), and IgM (IgM88; Biogenex, Fremont, CA).

Flow cytometric analysis of blood lymphocytes and B cell subsets

Absolute counts of blood CD4 and CD8 T cells, CD16/56+ natural killers cells, and CD19+ B cells were obtained with a diagnostic lyse-no-wash protocol. Eight-color flow cytometric immunophenotyping was performed as described previously to detect transitional, naive mature, CD21lowCD38low, plasma cell and memory B cell subsets (Figure 2) (24) and to detect CXCR3 expression levels using monoclonal antibodies against CD24-PB (ExBio), CD45-PO (Invitrogen, Carlsbad, CA), IgM-FITC, IgD-PE, IgG-PE, IgA-PE (all goat polyclonal from SBA, Washington, DC), CD19-PerCP-Cy5.5, CD21-PE-Cy7, CD27-APC, CD183-APC, CD38-APC-H7 and IgD-biotin (all from

BD Biosciences, Franklin Lakes, NJ) (24). Biotinylated antibodies were detected with Streptavidin PE-Cy7 (eBioscience, San Diego, CA).

Intracellular cytokine staining were performed on post-Ficoll blood mononuclear cells after 5 hours of in vitro activation. Cells were incubated with 50 μg/ml LPS, 1.5 μg/ml PMA (Sigma-Aldrich, St. Louis, MO), and 3 μg/ml ionomycin (Sigma-Aldrich); with LPS, 10 μg/ml anti-CD40 (Bioceros BV, Utrecht, The Netherlands), and 10 μg/ml IL-4 (U-Cytech, Utrecht, The Netherlands); or with 10 μg/ml PAM3CSK4, anti-CD40, and IL-4. After 1 hour of incubation, 10 μg/ml Golgi-stop (BD Biosciences) was added. Additional antibodies used for flow cytometry were IL-10-PE, TNF-α-PE-Cy7, CD19-HorV500 (all from BD Biosciences), IFN-γ-PE-Cy7, TGF-β-PE-Cy7 (both from BioLegend, San Diego, CA), IL-6-PE (eBioscience), and CD69-PE (Serotech, Kidlington, UK).

Quantification of serum immunoglobulin levels

IgG, IgA, and IgM serum levels were measured with an immunoturbidimetric method (Hitachi Analyzer; Roche, Basel, Switzerland). IgG and IgA subclasses were determined using the immunonephelometric method (Sanquin, Amsterdam, The Netherlands).

Vaccination responses

Responses to T cell-dependent influenza A/H1N1 (2007), A/H3N2 (2007) and B/Brisbane (Agriflu), and influenza A/H1N1 (2009) (Focetria; both from Novartis Pharma, Basel, Switzerland) vaccines were measured before and 4 weeks after vaccination with a standard hemagglutination-inhibition (HI) assay (28). A 4-week postvaccination HI titer of at least 1:40 combined with a more than fourfold increase in titer was defined successful (29, 30). Specific IgG and IgA levels were determined before and 4 weeks after vaccination with T cell-independent Streptococcus pneumoniae (Pneumo23) and serum levels of Haemophilus influenzae type B antigens. Adequate pneumococcal response was defined as more than fourfold increase over baseline or postimmunization titer values of more than 0.35 μg/ml for at least four of the six serotypes (28). H. influenzae vaccination was considered effective with an antibody concentration greater than 1μg/ml (31).

Amplification and sequence analysis of immunoglobulin heavy-chain transcripts

IGA and IGG transcripts were amplified from B cells in thawed mononuclear cells of patients with sarcoidosis and control subjects, cloned into pGEM-T easy vector (Promega, Madison, WI), and sequenced on an ABIPRISM

3130XL (Applied Biosystems, Carlsbad, CA) as described previously (24). Obtained sequences were analyzed with the IMGT database (http://imgt.cines.fr/) to assign V, D and J genes; to identify somatic mutations and to characterize the complementarily determining region (CDR)3 (32). IgA and IgG subclasses were determined using the immunoglobulin heavy chain (IGH) reference sequence (NG_001019).

Statistics

Statistical analyses were performed using the Mann-Whitney test (SPSS v18.0). A P value less than 0.05 was considered statistically significant.

Results

Patients

In this study, 32 patients with biopsy-confirmed sarcoidosis (13 male and 19 female) were included (mean age, 48 yr; range, 19-78 yr). Patients 1 through 28 had not received immunosuppressive drugs for at least 6 months, and patients 29 through 32 received infliximab (Table 1). The average duration of disease at study inclusion was 6.3 years (range, 1- 31 yr), and most patients had multiple organ involvement. Mean values of serum levels of angiotensin converting enzyme, B cells, CD4+ T cells, CD8+ T cells, and natural killer cells were within normal ranges for patients not receiving immunosuppressive drugs (Table 1).

Large numbers of B cells surround granulomas in patients with sarcoidosis

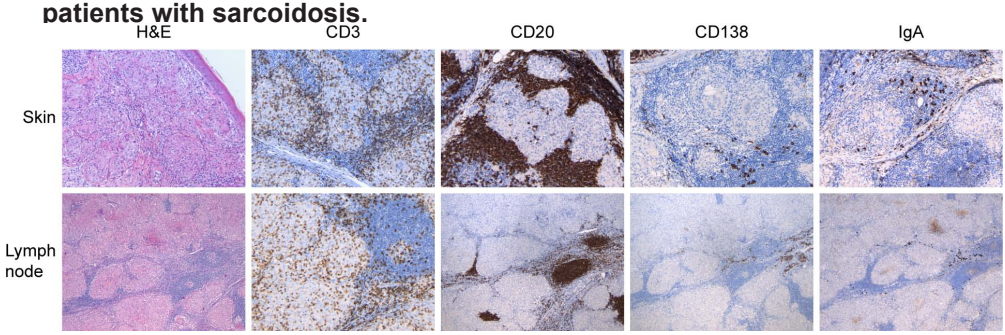
Haematoxylin and eosin-stained tissue biopsies of the granulomas did not show signs of necrosis. To study the presence of B cells in granulomas and their position in relation to T cells, several tissue biopsies were studied with immunohistochemistry. T cells that stained positive for CD3, CD4, CD5 and CD8 were easily detectable in all samples and were mainly located inside the granulomas (Figure 1). B cells were observed in all tissues. However, these were located in different areas as compared with T cells. B cells were predominantly located in the vicinity of the granulomas and in 5 of the 17 patients also inside the granulomas (Figure 1). These concerned mostly CD20+, CD79a+, and PAX5+ B lymphocytes, but also CD138+ plasma cells were found (Figure 1). The latter mostly produced IgA, followed by IgG and IgM (Figure 1). These patterns were comparable between the various tissues, including skin samples in which IgA plasma cells are not normally found (33).

Thus, the consistent presence of large numbers of B cells surrounding granulomas is suggestive of their involvement in the pathogenesis of sarcoidosis.

Abnormal naive and memory B cell subset distributions in blood of sarcoidosis patients

To study the peripheral B-cell compartment in more detail, we performed extensive immunophenotyping of blood B cells in 28 patients and 28 age-matched healthy control subjects. There was no significant difference of total B-cell numbers (CD19+) between these groups.

Figure 1. Large perigranuloma B cell infiltrates in affected tissue of patients with sarcoidosis.



Representative images of staining with hematoxylin and eosin (H&E), CD3, CD20, CD138, and IgA of a skin and a lymph node of the neck biopsy from patients with sarcoidosis. Representative pictures of skin and lymph node of patients with sarcoidosis are shown.

Within the CD19+ B-cell compartment, we distinguished two naive populations, CD21low anergic B cells, six memory B-cell subsets, and plasma cells (Figure 2) (23). CD24hiCD38hi transitional and naïve mature B cells were similar in patients compared with healty control subjects (Figure 3A). Of the six memory B-cells subsets, natural effector B cells, CD27+IgM+, CD27+IgG+, and CD27+IgA+ memory B cells were significantly reduced compared with healthy control subjects (all $P < 0.0001$) (Figure 3A). Although CD27-IgG+ memory B cells were similar, T cell-independently derived CD27-IgA+ B cells were significantly increased in patients with sarcoidosis ($P = 0.002$) (Figure 3A). Independent of disease duration, the fraction of CD21lowCD38low anergic B cells and circulating plasma cells was reduced compared with healthy control subjects (Figure 3C and 3D) (8).

Table 1. Patient characteristics.

Patient	Gender	Age (yr)	Disease Duration	Localization	Previous Systemic Medication	ACE (U/L)	B cells (Cells/µL)	$CD4^+$ T cells (Cells/µL)	$CD8^+$ T cells (Cells/µL)	NK cells (Cells/µL)
1	M	39	4	Lung	CC, HCQ	67	129	**190**	**168**	252
2	F	37	3	Lung	CC, HCQ, MTX	36	176	547	224	**67**
3	F	47	4	Lung, eye	CC, HCQ, MTX	12	310	584	397	259
4	M	57	2	Lung, eye	HCQ	46	168	**374**	**174**	165
5	F	38	2	Lung, eye	HCQ	**95**	**508**	620	200	279
6	F	34	4	Lung, eye	CC, MTX	**77**	183	526	393	271
7	M	76	1	Lung, eye	HCQ	21	136	989	360	117
8	F	60	2	Lung, eye, skin	HCQ	62	136	**387**	**76**	**498**
9	F	34	4	Lung, eye, skin	CC, HCQ, MTX	30	**573**	696	364	123
10	M	35	2	Lung, eye, skin	CC, HCQ	**114**	232	**235**	**188**	214
11	F	63	1	Lung, eye, skin	HCQ	52	111	594	**124**	141
12	M	40	8	Lung, eye, skin, liver	CC, HCQ	31	**45**	510	438	137
13	V	60	13	Lung, eye, skin, joints	HCQ, IFX	48.6	127	576	**94**	109
14	F	35	3	Lung, skin	None	50	**475**	481	**72**	**80**
15	F	58	5	Lung, skin	HCQ	66	343	453	**42**	220
16	F	37	2	Lung, skin	HCQ	34	**408**	790	297	100
17	M	28	1	Lung, skin, liver, joints	CC, HCQ	**145**	**407**	617	**183**	127
18	M	31	1	Lung, skin, joints	None	**78.1**	**574**	**1722**	395	217

Patient	Gender	Age (yr)	Disease Duration	Localization	Previous Systemic Medication	ACE (U/L)	B cells (Cells/µL)	$CD4^+$ T cells (Cells/µL)	$CD8^+$ T cells (Cells/µL)	NK cells (Cells/µL)
19	V	58	22	Lung, skin, joints, NS	CC, HCQ, MTX, IFX	53.5	**73**	**103**	**179**	**26**
20	M	57	31	Lung, NS	CC, HCQ	51	358	884	584	176
21	M	41	6	Lung, NS	CC	34.0	**16**	**165**	**28**	169
22	F	69	14	Eye, liver	CC, HCQ, MTX	25	107	932	**132**	245
23	M	19	1	Eye, inguinal glands	None	30	168	832	466	102
24	F	72	2	Eye, skin	CC, HCQ	42	136	**268**	347	250
25	F	71	1	Skin, NS	None	45	116	626	468	229
26	M	67	24	Skin, NS	CC, HCQ	56.9	150	**245**	**103**	166
27	M	34	1	Heart	None	45	132	776	240	147
28	F	41	21	Larynx	CC	23	311	1077	247	201
29*	V	42	9	Lung, eye	CC, MTX	53.7	244	824	349	249
30*	M	39	3	Lung, skin	HCQ, MTX	24.1	**466**	**362**	258	**86**
31*	V	60	3	Lung, joints	CC	56.3	175	**1560**	267	362
32*	V	78	2	Lung, eye, joints	HCQ, MTX	35.2	206	519	291	159

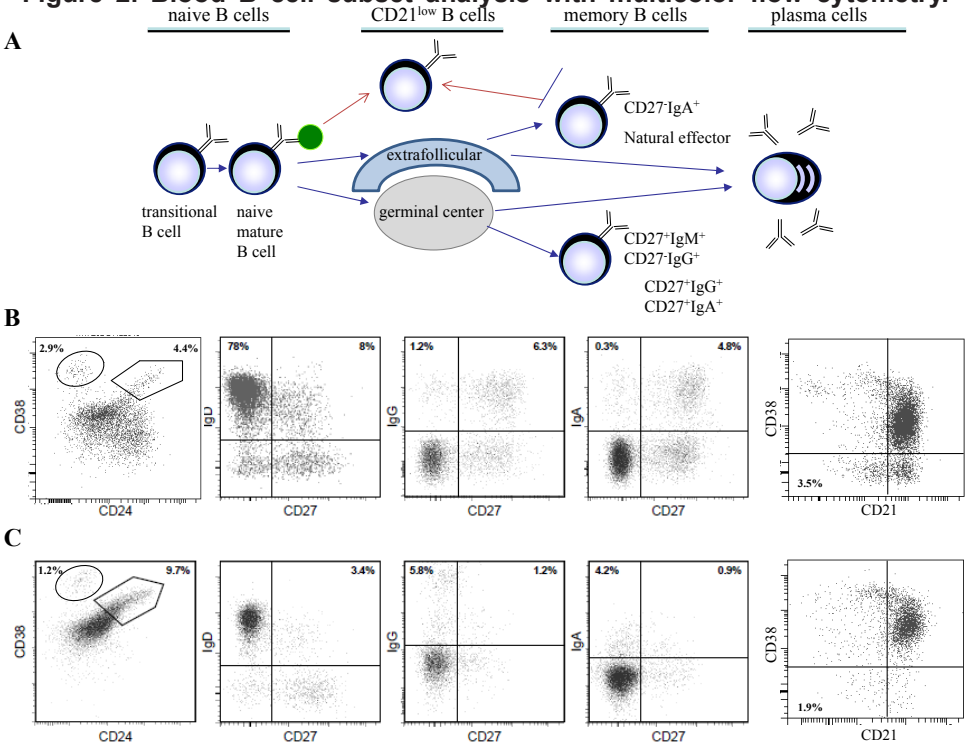
Definition of abbreviations: F = female; M = male; NS = nervous system; CC = corticosteroids; HCQ = hydroxychloroquine; MTX = methotrexate; IFX = infliximab; ACE = angiotensin converting enzyme.

Normal values: ACE, 12-68 U/L ; B cells, 100-400 cells/µL; CD4+ T cells, 400-1,300 cells/µL; CD8+ T cells, 200-700 cells/µL; NK cells, 100-400 cells/µL.

Bold = abnormal values ; * = patient is currently treated with infliximab.

Together, these data indicated that despite normal to high output of naive B cells from bone marrow, patients do not show increased anergy but appear impaired in generation or maintenance of normal numbers of most subsets of circulating memory B cells and plasma cells.

Figure 2. Blood B cell subset analysis with multicolor flow cytometry.

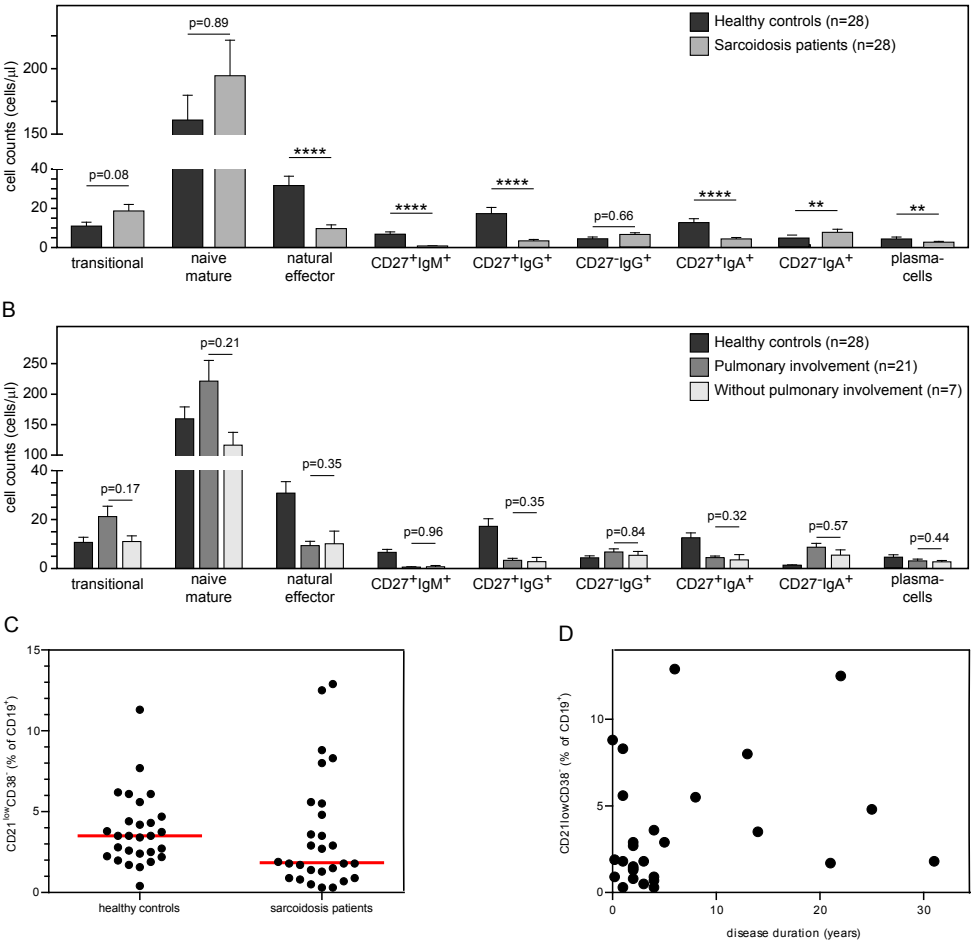


(A) Schematic representation of B cell maturation indicating the subsets that can be detected in peripheral blood. Transitional B cells are recent bone marrow emigrants and will develop into naïve mature B cells. CD27-IgA+ and natural effector B cells can be derived independently from T cell help. CD27+IgM+ and CD27-IgG+ memory B cells resembled those of primary germinal center cells, whereas CD27-IgG+ and CD27+IgA+ memory B cells has increased proliferation and somatic hypermutation levels suggestive of further maturation in consecutive germinal center response (22). Representative dot plots of flow cytometric analysis of a healthy control subject (B) and a patient with sarcoidosis (C) are shown. All B cell subsets are determined within the CD19+ lymphogate.

To determine whether distinct tissue involvement affected the B-cell compartment differently, blood B-cell subset numbers were compared between patients with (n = 21) and without pulmonary involvement (n = 7) (Figure 3B). Whereas transitional B cells only were increased in patients with pulmonary involvement, the patterns of circulating CD21low CD38low B cells, memory B-cell

subsets and plasma cells were similar between the two groups. Thus, most effects on the peripheral B-cell compartment are most likely unrelated to pulmonary involvement in patients with sarcoidosis.

A **Figure 3. Blood B cell subsets are affected in sarcoidosis.**



(A) Transitional and naïve mature B cells were normally present in patients with sarcoidosis. Significantly reduced numbers of natural effector B cells, CD27+IgM+, CD27+IgG+, and CD27+IgA+ memory B cells and plasma cells were found in patients with sarcoidosis as compared with healthy control subjects, whereas CD27-IgG+ were normally present and CD27-IgA+ B cells were significantly increased. (B) All abnormalities found in A were similar between pulmonary and without pulmonary sarcoidosis ($P > 0.17$). (C) CD21lowCD38low anergic B cells of patients were significantly reduced as compared with healthy control subjects. (D) No effect was seen of disease duration of sarcoidosis on the frequencies of CD21lowCD38low anergic B cells. Values are expressed as cell counts (cells/µl) ± SEM. Statistical analysis was performed with the Mann-Whitney test. *P <0.05. **P <0.01. **** P <0.0001.

Normal total serum immunoglobulin levels and antigen-specificimmunoglobulin responses in patients with sarcoidosis.

The impaired humoral immune response in patients with CVID is related to reduced numbers of circulating memory B cells (18). Comparably low numbers of memory B cells in the studied patients prompted us to analyze total serum immunoglobulin levels and immunoglobulin responses after vaccinations with T cell-dependent and T cell-independent antigens. In contrast to patients with CVID, normal levels of total serum IgG, IgA and IgM levels, as well as IgG and IgA subclass levels were observed in all tested patients (Table 2). Thus, terminal differentiation of B cells into immunoglobulin-producing plasma cells appeared unaffected in patients. The vaccination response of all patients for T cell-dependent seasonal flu (Agriflu 2009-2010) and Mexican flu (Focetria) antigens were within the normal range. In addition, in all patients but one, normal responses to T cell-independent Streptococcus pneumoniae and H. influenzae type B antigens were generated. One patient (patient 16; Table 1) did not respond adequately to S. pneumonia but responded normally to H. influenza, and the B-cell subset distribution of this patient was comparable with the whole group. Thus, patients with sarcoidosis are capable of mounting normal immunoglobulin responses with or without assistance of T cells.

Increased molecular signs of antibody maturation in patients with sarcoidosis

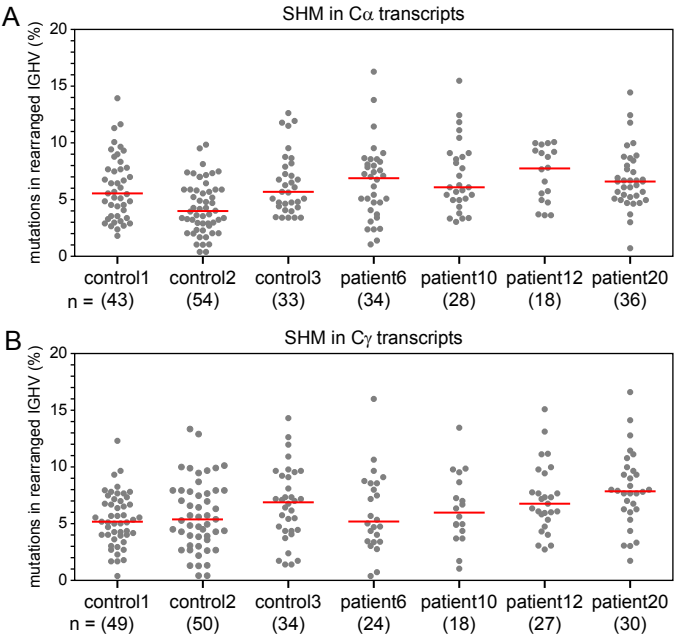
Despite the reduced numbers of circulating memory B cells, immunoglobulin responses were normal in patients with sarcoidosis. To determine whether this applies to the molecular levels, we studied somatic hypermutations (SHMs) and IgA and IgG subclass use in sequenced IGH transcripts of B cells from our patients and three healthy control subjects. Transcripts of all four patients showed increased signs of antigen maturation, with high frequencies of SHMs (Figure 4A and 4B) and accumulation of replacement mutations in CDRs compared with healthy control subjects (Figure 4C and 4D).

To study antibody maturation in more detail, we determined the IgA and IgG subclasses of the amplified transcripts (Figure 5B). IgA transcripts showed similar distributions between patients with sarcoidosis and control subjects with slightly more IgA1 usage than IgA2. Despite this, SHM levels of IgA1 and IgA2 transcripts were increased in patients with sarcoidosis as compared with healthy control subjects (Figure 5C).

Table 2. Immunoglobulin levels and vaccination responses.

	Before Vaccination Mean (Range)	After Vaccination*- Mean (Range)	Control Values
Serum immunoglobulin levels (n = 11)			
Total IgM, g/L	1.1 (0.6-2.0)	-	0.5-2.3
Total IgA, g/L	2.7 (1.5-3.7)	-	0.8-3.9
Total IgA1, g/L	1.9 (1.0-2.4)	-	0.6-2.4
Total IgA2, g/L	0.5 (0.2-0.6)	-	0.1-0.6
Total IgG, g/L	12 (7.0-15.7)	-	7.0-16.0
Total IgG1, g/L	9.1 (5.3-11.3)	-	4.9-11.4
Total IgG2, g/L	2.9 (1.5-6.0)	-	1.5-6.4
Total IgG3, g/L	0.6 (0.2-1.1)	-	0.2-1.1
Total IgG4, g/L	0.9 (0.4-1.4)	-	0.1-1.4
Vaccine			
Influenza A/H1N1 (2007) (n = 11)	88 (5-400)	371 (80-1280)	-
Influenza A/H3N2 (2007) (n = 11)	50 (5-300)	375 (20-1760)	-
Influenza B/Brisbane (2008) (n = 11)	28 (5-150)	347 (20-1280)	-
Influenza A/H1N1 (2009) (n = 8)	52 (5-130)	432 (195-960)	-
Streptococcus pneumoniae IgG (n = 12)			
Serotype 1, µg/ml	1.1 (0.10-5.24)	2.2 (0.1-6.32)	>0.35
Serotype 3, µg/ml	0.4 (0.11-8.50)	2.4 (0.25-8.50)	>0.35
Serotype 4, µg/ml	0.3 (0.10-0.42)	1.9 (0.10-4.07)	>0.35
Serotype 5, µg/ml	1.1 (0.10-4.31)	2.6 (0.10-8.63)	>0.35
Serotype 9, µg/ml	0.7 (0.10-1.94)	2.6 (0.13-7.77)	>0.35
Serotype 23, µg/ml	0.4 (0.10-1.36)	2.4 (0.10-8.10)	>0.35
Streptococcus pneumonia IgA (n = 10)			
Serotype 1, µg/ml	0.17 (0.06-0.66)	1.31 (0.63-1.42)	>0.35
Serotype 3, µg/ml	0.75 (0.00-1.75)	1.99 (0.87-4.30)	>0.35
Serotype 4, µg/ml	0.08 (0.00-0.18)	1.10 (0.34-1.18)	>0.35
Serotype 5, µg/ml	0.12 (0.04-0.20)	0.76 (0.35-1.24)	>0.35
Serotype 9, µg/ml	0.09 (0.00-0.18)	1.15 (0.43-1.70)	>0.35
Serotype 23, µg/ml	0.11 (0.00-0.38)	0.50 (0.06-1.29)	>0.35
Haemophilus influenza type B (n = 12), µg/ml	1.5 (0.3-7.1)	44.0 (4.2-35.0)	>1.0

Figure 4. Extensive signs of antigen maturatation in IgG and IgA transcripts of sarcoidosis patients.



(A and B) Transcripts of four representative patients were compared with three control subjects. All four patients showed high frequencies of somatic hypermutation (SHM) (n represents the total number of transcripts). (C and D) Similar accumulation of replacement mutations in CDR1 and CDR2 regions between patients with sarcoidosis and age-matched healthy control subjects. IGHV = immunoglobulin heavy chain variable.

IgG transcripts showed equal use of IgM-proximal IgG1 and IgG3 (58%) and IgM-distal IgG2 and IgG4 (42%) in patients with sarcoidosis and healthy control subjects (Figure 5B). IgG1 and IgG2 transcripts of patients with sarcoidosis showed increased levels of SHM, comparable to the IgA transcripts. Thus, although IgA class switching appears unaffected, increased levels of both SHMs and increased immunoglobulin-class switching to downstream IgG regions suggest that B-cell memory in patients with sarcoidosis is generated in prolonged or additional immune responses. Therefore, despite reduced numbers of circulating memory B cells and plasma cells, patients with sarcoidosis are fully capable of generating functional humoral immunity with molecular signs of antigen maturation.

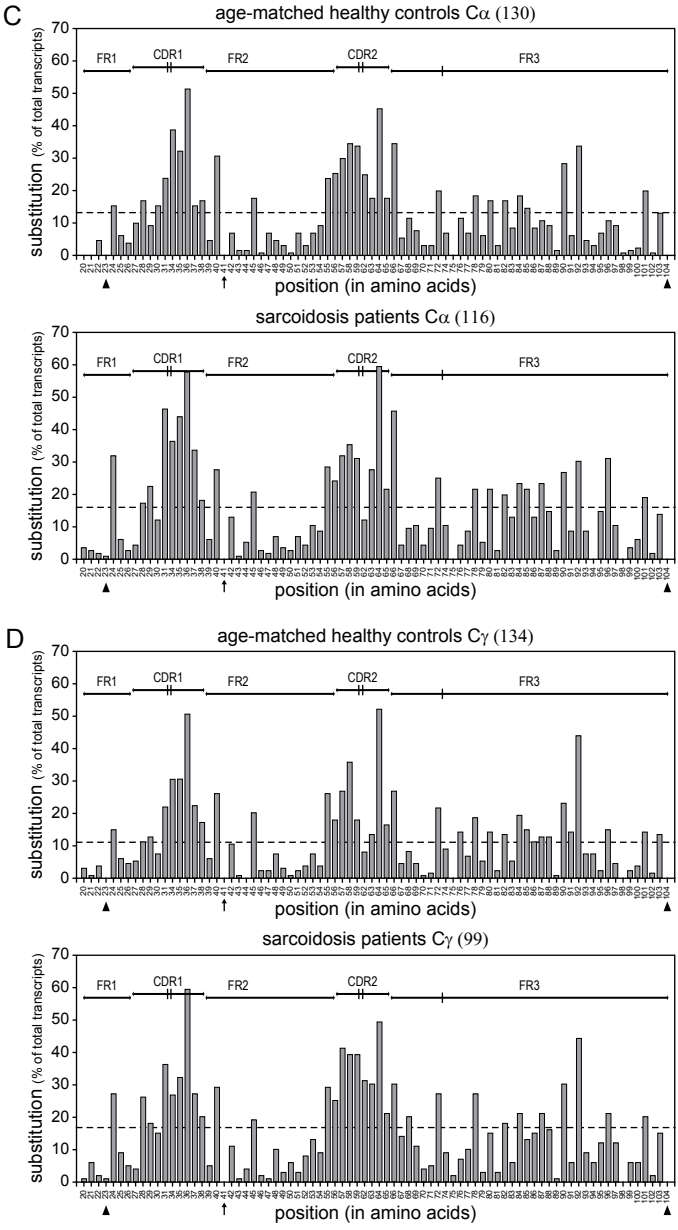
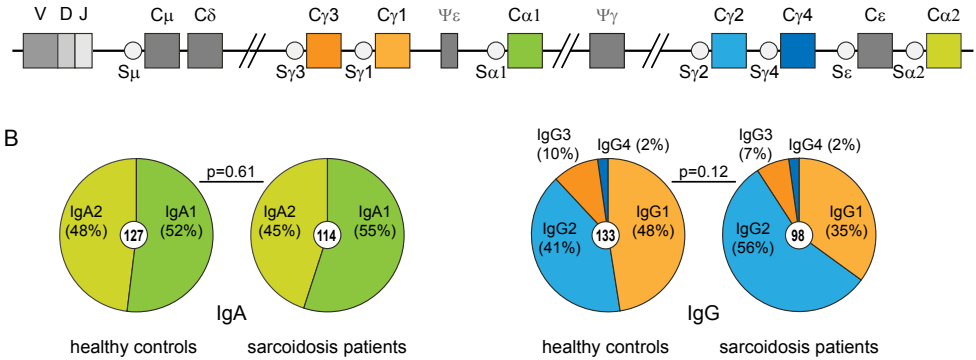
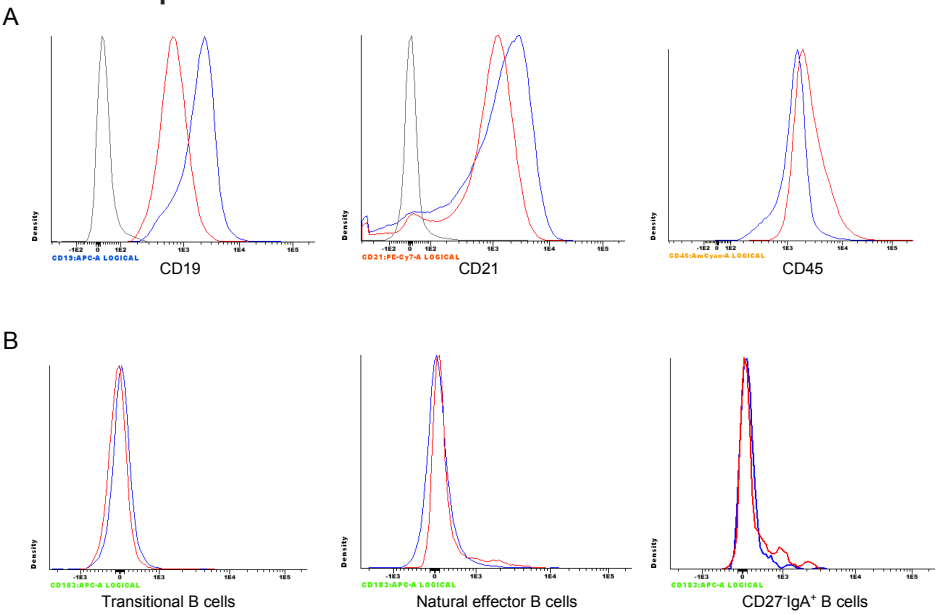

C
age-matched healthy controls Cα (130)
sarcoidosis patients Cα (116)
D
age-matched healthy controls Cγ (134)
sarcoidosis patients Cγ (99)
FR1
CDR1
FR2
CDR2
FR3
substitution (% of total transcripts)
position (in amino acids)

Figure 5. Increased Ig class switching to downstream IgG subclasses in patients with sarcoidosis.



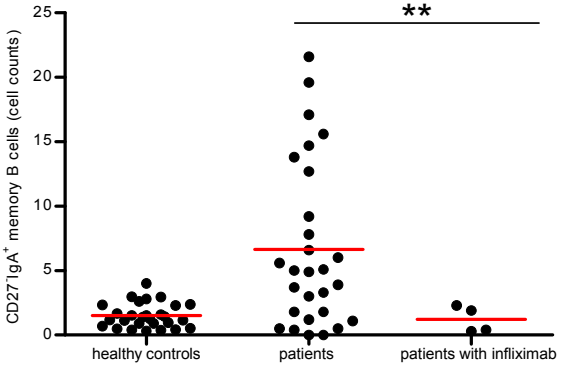
(A) Schematic representation of the constant region of the human immunoglobulin heavy-chain (IGH) locus. (B) Distribution of IgA and IgG receptor subclass use in IGH rearrangements of class-switched memory B cell subsets is shown. IgA transcripts showed equal IgA1 and IgA2 usage in patients with sarcoidosis as compared to control subjects. IgG transcripts from control subjects showed a trend of dominant use of IgG1 and IgG3 (58%) over IgG2 and IgG4 as compared with SP (42% IgG1 and IgG3). Total number of analyzed sequences is indicated in the center of each plot. (C) Somatic hypermutation levels of IgA1, IgA2, IgG1 and IgG2 transcripts were increased in patients with sarcoidosis as compared with control subjects. Statistical analysis was performed with the Mann-Whitney test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 6. Sarcoid B cells showed elevated expression of CD19, CD21 and decreased expression of CD45 with normal CXRC3 levels.



(A) CD19, CD21, and CD45 expression in a representative patient (red), a healthy control (blue), and negative control subject (gray). Significant lower expression was found in a patient with sarcoidosis of CD19 and CD20. CD45 expression was slightly elevated in patients with sarcoidosis. (B) CXCR3 expression in a representative patient (red) and a healthy control subject (blue) showed no difference in expression on transitional, natural effector, or CD27-IgA+ B cells.

Figure 7. Normalization of CD27-IgA+ memory B cells in patients with sarcoidosis treated with a TNF-α blocker.



CD27-IgA+ memory B cells were compared between patients with sarcoidosis without treatment and treatment with infliximab (a TNF-α blocker). The CD27-IgA+ memory B cell population normalized in patients who were treated with infliximab.

No production of TNF-α and increased levels of IFN-γ by stimulated B cells

To determine if the distinguished B-cell differentiation in patients with sarcoidosis was directly involved in local inflammatory processes in sarcoidosis, functional analysis in five patients (patients 13, 15, 18, 19, and 22) (Table 1) was performed. Sarcoid B cells showed decreased expression of CD19 (mean fluorescence intensity [MFI], 2,335) and CD21 (MFI 2,561) and elevated expression of CD45 (MFI 1,300) as compared with healty control subjects (Figure 6A). CXRC3 levels of patients with sarcoidosis and healthy control subjects were similar in all B cells (Figure 6B). B cells of patients with sarcoidosis were stimulated in three different cultures (culture 1: LPS, PMA, ionomycin; culture 2: LPS, CD40L, IL-4; and culture 3: PAM3CSK4, CD40L, IL-4). After stimulation, no significant difference between patients with sarcoidosis and healthy control subjects in the levels of expression of TNF-α, TGF-β, IFN-γ, IL-6, and IL-10 was found.

Population of CD27-IgA+ memory B cells normalized in patients currently treated with TNF-α blockers

To study the effect of TNF-α blockers on B cells, we determined the B-cell differentiation in four patients (patients 29-32) (Table 1) who were currently treated with a TNF-α blocker (i.e., infliximab) and compared them with healthy control subjects. All B-cell subsets were similar except for CD27-IgA+ memory B cells. In patients with sarcoidosis, this B-cell population was significantly increased as compared with healthy control subjects. In patients currently treated with TNF-α blockers, this population normalized ($P = 0.045$) (Figure 7). Thus, the elevated population of CD27-IgA+ memory B cells in patients with sarcoidosis normalizes after treatment with a TNF-α blocker.

Discussion

In this study, we analyzed distribution patterns and cellular and molecular characteristics of B cells of patients with sarcoidosis. The observations suggest skewing of memory B cells toward granulomatous tissue. This and our observation of increased circulating transitional B cells and normal immunoglobulin responses to vaccination but increased molecular signs of antibody maturation indicates an overactive B-cell response that might play a role in disease pathophysiology of sarcoidosis.

The presence of B cells in or close to granulomas of patients with sarcoidosis may be of clinical significance. To our knowledge, the only report of B cells in sarcoid granulomas was published in the early 1990s and was

restricted to lung and lymph node biopsies (12). Despite the reported high numbers of B cells around granulomas, no studies have been reported since. Hypergammaglobulinemia can be found in patients with sarcoidosis (10). Thus, it is conceivable that immunohistochemical studies were focused on staining against immunoglobulin secreting plasma cells and not against B cells (10). Our findings revealed, apart from low plasma cell presentation, large numbers of B cells in granulomatous tissue of various affected organs, suggesting that these are disease related and not organ dependent.

Detailed analysis of naive B cells in blood revealed increased numbers of transitional B cells in line with a previous study (34). Because transitional B cells are recent bone marrow emigrants, B-cell output from bone marrow does not appear impaired in patients with sarcoidosis. Decreased frequencies of anergic CD21lowCD38low B cells were found in our study. The reduction of CD21lowCD38low B cells contradicts published work by Lee and colleagues, who described low levels of CD21 expression on B cells and increased anergy in patients with chronic sarcoidosis with disease duration of more than six years (8). Of the 28 studied patients, only seven have been diagnosed more than six years before. However, no increased anergy was observed in these patients. Apparently, our patients do not display increased anergy in their B-cell compartment.

In line with previous observations, we found reduced levels of CD19 and CD21 and elevated CD45 levels on B cells of our patients with sarcoidosis (8). CD19 and CD20 are positive regulators of B-cell receptor signal transduction (35). In fact, human CD19 and CD20 deficiencies result in a higher threshold for activation and thus hyporesponsiveness to transmembrane stimulation (36, 37). Reduced CD19 or CD20 expression can result in reduced responsiveness of these lymphocytes.

The blood memory B-cell compartment is clearly affected in patients with sarcoidosis, with significantly reduced numbers of natural effector B cells and T cell-dependent CD27+IgM+, CD27+IgG+, and CD27+IgA+ memory B-cell subsets. An overall decrease of CD27+ memory B cells has been reported recently in severe chronic sarcoidosis (8, 34). However, our more detailed analysis reveals that this is mostly due to a decrease of mainly T cell-dependent B-cell memory in sarcoidosis.

In contrast to the CD27+ memory B cells, T cell-independent CD27-IgA+ memory B cells are increased in the studied patients. Furthermore, plasma cells in granulomatous tissue produce mostly IgA. These findings are especially interesting because IgA-producing plasma cells produce TNF-α, one of the key cytokines in the pathogenesis of sarcoidosis (7, 38).

However, after stimulation of CD27-IgA+ memory B cells with LPS+PMA, CD40L, or PAM3CSK4, we did not observe TNF-α expression in these cells. Because these cells did not produce cytokines after a 5-hour stimulation with these broad stimuli, we did not pursue stimulation experiments with specific s arcoid antigens. Infiliximab is a TNF-α blocker and is used in the treatment of sarcoidosis (3). We found that treatment with a TNF-α blockers normalizes the population of CD27-IgA+ memory B cells. Thus, circulating numbers of CD27-IgA+ memory B cells could be a marker of sarcoidosis activity.

Despite the strongly reduced number of memory B cells in blood, patiens with sarcoidosis show only slightly reduced numbers of circulating plasma cells, normal serum immunoglobulin levels, and normal responses to vaccinations. This is in line with previous studies demonstrating normal vaccination responses to influenza vaccinations and the clinical observation that untreated patients display no increased risk for infections (39, 40). Therefore, it is unlikely that the decreased numbers of memory B cells are the result of impaired B-cell responses.

Detailed molecular analysis of IgA and IgG memory B cells in this study demonstrate increased SHM levels and increased immunoglobulin class switching to downstream IgG subclasses in patients with sarcoidosis compared with control subjects. These molecular patterns suggest that memory B cells in sarcoidosis have been exposed to prolonged B-cell stimulation or consecutive responses (19, 41, 42). Thus, it is unlikely that patients have defects in the generation of B-cell memory but rather have involvement and migration of antigen-experienced B cells towards granulomatous tissue. Further evidence for chronic B-cell stimulation is the increased risk for patients to develop a malignant lymphoma (sarcoidosis-lymphoma syndrome) (41, 43, 44). These lymphomas are predominantly of B-cell origin. It is thought that these B cells undergo malignant transformation after chronic stimulation (45).

The abnormalities in the peripheral B-cell compartment in sarcoidosis with increased numbers of transitional B cells and decreased memory B-cell numbers is similar to patients with granulomatous CVID (18, 46-48). However, in contrast to patients with CVID, vaccination responses, circulating plasma cell numbers, and serum immunoglobulin levels and degrees of antibody maturation were not severely affected in patients with sarcoidosis. In contrast to patients with CVID who do have circulating B cells, granuloma formation has not been reported in patients with complete B-cell deficiency (e.g., with X-linked agammaglobulinemia) (49). Additionally, in animal models of T-cell-deficient mice, it has been shown that B cells are

crucial for noninfectious granuloma formation (50). Thus B cells appear to be essential for granuloma formation in antibody-deficient patients and in patients with sarcoidosis.

In conclusion, our extensive analysis of B cells in patients with sarcoidosis demonstrates significant B-cell infiltrates in granulomatous tissue, whereas blood memory B-cell compartment is reduced in size (despite normal immunoglobulin responses) and skewed to locally produced CD27-IgA+ B cells. These data are indicative of direct involvement of B cells in local inflammatory processes in granulomatous tissue of patients with sarcoidosis with CD27-IgA+ B cells as a potential marker for disease activity of sarcoidosis. Therefore, our study provides a rationale for a systematic study on B-cell ablative therapy (e.g., with rituximab) in patients with sarcoidosis.

References

1. Hinze CH, Colbert RA. B-cell depletion in Wegener's granulomatosis. Clin Rev Allergy Immunol. 2008;34:327-379.
2. Nakken B, Munthe LA, Konttinen YT, Sandberg AK, Szekanecz Z, Alex P, Szodoray P. B-cells and their targeting in rheumatoid arthritis--current concepts and future perspectives. Autoimmun Rev. 2011;11:28-34.
3. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
4. Morgenthau AS, Iannuzzi MC. Recent advances in sarcoidosis. Chest. 2011;139:174-182.
5. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med. 2011;183:573-581.
6. Iannuzzi MC, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. JAMA. 2011;305:391-399.
7. Kamphuis LS, Lam-Tse WK, Dik WA, van Daele PL, van Biezen P, Kwekkeboom DJ, Kuijpers RW, Hooijkaas H, van Laar JA, Bastiaans J, Baarsma GS, van Hagen PM. Efficacy of adalimumab in chronically active and symptomatic patients with sarcoidosis. Am J Respir Crit Care Med. 2011;184:1214-1216.
8. Lee NS, Barber L, Akula SM, Siqounas G, Kataria YP, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. Clin Vaccine Immunol. 2011;8:1306-1316.
9. Daniele RP, McMillan LJ, Dauber JH, Rossman MD. Immune complexes in sarcoidosis: a correlation with activity and duration of disease. Chest. 1978;74:261-264.
10. Hunninghake GW, Crystal RG. Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis. Site of increased antibody production and role of T lymphocytes. J Clin Invest. 1981;67:86-92.
11. Weinberg I, Vasiliev L, Gotsman I. Anti-dsDNA antibodies in sarcoidosis. Semin Arthritis Rheum. 2000;29:328-331.
12. Fazel SB, Howie SEM, Krajewski AS, Lamb D. B lymphocytes accumulations in human pulmonary sarcoidosis. Thorax. 1992;47:964-967.
13. Gottenberg JE, Guillevin L, Lambotte O, Combe B, Allanore Y, Cantagrel A, Larroche C, Soubrier M, Bouillet L, Dougados M, Fain O, Farge D, Kyndt X, Lortholary O, Masson C, Moura B, Remy P, Thomas T, Wendling D, Anaya JM, Sibilia J, Mariette X; Club Rheumatismes et Inflammation (CRI). Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases. Ann Rheum Dis. 2005;64:913-920.
14. Belkhou A, Younsi R, El Bouchti I, El Hassani S. Rituximab as a treatment alternative in sarcoidosis. Joint Bone Spine. 2008;75:511-512.
15. Dasilva V, Breuil V, Chevallier P, Euller-Ziegler L. Relapse of severe sarcoidosis with an uncommon peritoneal location after TNF alpha blockade. Efficacy of rituximab, report of a single case. Joint Bone Spine. 2010;77:82-83.
16. Bomprezzi R, Pati S, Chansakul C, Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. Neurology. 2010;75:568-570.
17. Artac H, Bozkurt B, Talim B, Reisli I. Sarcoid-like granulomas in common variable immunodeficiency. Rheumatol Int. 2009;30:109-112.

18. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, Vlkova M, Hernandez M, Detkova D, Bos PR, Poerksen G, von Bernuth H, Baumann U, Goldacker S, Gutenberger S, Schlesier M, Bergeron-van der Crussen F, Le Garff M, Debré P, Jacobs R, Jones J, Bateman E, Litzman J, van Hagen PM, Plebani A, Schmidt RE, Thon V, Quinti I, Espanol T, Webster AD, Chapel H, Vihinen M, Oksenhendler E, Peter HH, Warnatz K. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111:77-85.
19. Driessen GJ, van Zelm MC, van Hagen PM, Hartwig NG, Trip M, Warris A, de Vries E, Barendregt BH, Pico I, Hop W, van Dongen JJ, van der Burg M. B-cell replication history and somatic hypermutation status identify distinct pathophysiological backgrounds in common variable immunodeficiency. Blood. 2011;118:6814-6823.
20. Zhang M, Wang Z, Graner MW, Yang L, Liao M, Yang Q, Gou J, Zhu Y, Wu C, Liu H, Zhou B, Chen X. B cell infiltration is associated with the increased IL-17 and IL-22 expression in the lungs of patients with tuberculosis. Cell Immunol. 2011;270:217-223.
21. Gonzalez-Juarrero M, Turner OC, Turner J, Marietta P, Brooks JV, Orme IM. Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with Mycobacterium tuberculosis. Infect Immun. 2001;69:1722-1728.
22. Van Zelm MC, van der Burg M, de Ridder D, Barendregt BH, de Haas EF, Reinders MJ, Lankester AC, Révész T, Staal FJ, van Dongen JJ. Ig gene rearrangement steps are initiated in early human precursor B cell subsets and correlate with specific transcription factor expression. J Immunol. 2005;175:5912-5922.
23. Berkowska MA, van der Burg M, van Dongen JJ, van Zelm MC. Checkpoints of B cell differentiation: visualizing Ig-centric processes. Ann N Y Acad Sci. 2011;1246:11-25.
24. Berkowska MA, Driessen GJ, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, Cerutti A, He B, Biermann K, Lange JF, van der Burg M, van Dongen JJ, van Zelm MC. Human memory B cells originate from three distinct germinal center-dependent and –independent maturation pathways. Blood. 2011;118:2150-2158.
25. Kamphuis LSJ, van Zelm MC, de Haas EFE, Tse WK, van Laar JAM, van Daele PLA, Baarsma GS, van Hagen PM. B-cell development in sarcoidosis. [Poster]. EWIMID 2009 Cascais-Lisbon.
26. Kamphuis LSJ, van Zelm MC, de Haas EFE, Tse WK, van Laar JAM, van Daele PLA, van Hagen PM. B-cell maturation in a granulomatous disease. [Poster]. ESID 2010 Istanbul.
27. Kamphuis LSJ, van Zelm MC, van Hagen PM, de Haas EFE, Lam KH, Rimmelzwaan GF, van den Burg M, van Daele PLA, van Laar. Are B-cells emerging as key players in the pathogenesis of sarcoidosis? [Poster]. WASOG BAL 2011 Maastricht.
28. Vogtlander NP, Brown A, Valentijn RM, Rimmelzwaan GF, Osterhaus AD. Impaired response rates, but satisfying protection rates to influenza vaccination in dialysis patients. Vaccine. 2004;27:2469-2474.
29. European Committee for Proprietary Medicinal Products (1997). Note for guidance on harmonisation of requirements for Influenza vaccines. (CPMP/BWP/214/96). London: European Agency for the Evaluation of Medicinal Products.
30. Center for Biologics Evaluation and Research (May 2007). Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. Bethesda MD: Food and Drug Administration.
31. Advisory Committee for Immunization Practices. Advisory Committee for Immunization Practices Recommendations. http://www.cdc.gov/vaccines/pubs/ACIP-list.htm.
32. Lefranc MP. IMGT databases, web resources and tools for immunoglobulin and T cell receptor sequence analysis, http://imgt.cines.fr. Leukemia. 2003;17:260-266.

33. Saklayen MG, Schroeter AL, Nafz MA, Jalil K. IgA deposition in the skin of patients with alcoholic liver disease. J Cutan Pathol. 1996;23:12-18.
34. Saussine A, Tazi A, Feuillet S, Rybojad M, Juillard C, Bergeron A, Dessirier V, Bouhidel F, Janin A, Bensussan A, Bagot M, Bouaziz JD. Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10-producing regulatory B cells and high BAFF levels. PLoS One. 2012;7:e43588.
35. Hasler P, Zouali M. B cell receptor signaling and autoimmunity. FASEB J. 2001;15:2085-2098.
36. van Zelm MC, Reisli I, van der Burg M Castaño D, van Noesel CJ, van Tol MJ, Woellner C, Grimbacher B, Patiño PJ, van Dongen JJ, Franco JL. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006;354:1901-1912.
37. Thiel J, Kimmig L, Salzer U, Grudzien M, Lebrecht D, Hagena T, Draeger R, Völxen N, Bergbreiter A, Jennings S, Gutenberger S, Aichem A, Illges H, Hannan JP, Kienzler AK, Rizzi M, Eibel H, Peter HH, Warnatz K, Grimbacher B, Rump JA, Schlesier M. Genetic CD21 deficiency is associated with hypogammaglobulinemia. J Allergy Clin Immunol. 2012;129:801-810.
38. Fritz JH, Rojas OL, Simard N, McCarthy DD, Hapfelmeier S, Rubino S, Robertson SJ, Larijani M, Gosselin J, Ivanov II, Martin A, Casellas R, Philpott DJ, Girardin SE, McCoy KD, Macpherson AJ, Paige CJ, Gommerman JL. Acquisition of a multifunctional IgA+ plasma cell phenotype in the gut. Nature. 2011;481:199-203.
39. Girard N, Cottin V, Hot A, Etienne-Mastroianni B, Chidiac C, Cordier JF. Opportunistic infections and sarcoidosis. Rev Mal Respir. 2004;21:1083-1090.
40. Tavana S, Argani H. Gholamin S, Razavi SM, Keshtkar-Jahromi M, Talebian AS, Moqhaddam KG, Sepehri Z, Azad TM, Keshtkar-Jahromi M. Influenza vaccination in patients with pulmonary sarcoidosis: efficacy and safety. Influenza Other Respi Viruses. 2012;6:136-141.
41. Küppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005;4:251-262.
42. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia: BCR response and IGHV mutation. Nat Rev Cancer. 2010;10:37-50.
43. Papanikolaou IC, Sharma OP. The relationschip between sarcoidosis and lymphoma. Eur Respir J. 2010;36:1207-1219.
44. García Casares E, Mateo Soria L, García Melchor E. Mínguez Blasco S, Vall-Llobera Calmet F, Cañellas Oliver G, Holgado Pérez S. Sarcoidosis-lymphoma syndrome. Reumatol Clin. 2009;1:31-33.
45. Karakantza M, Matutes E, McLennan K, O'Connor NT, Srivastava PC, Catovsky D. Association between sarcoidosis and lymphoma revisited. J Clin Pathol. 1996;49:208-212.
46. Cunningham-Rundles C. Autoimmune manifestations in common variable immunodeficiency. J Clin Immunol. 2008;Suppl 1:S42-45.
47. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol. 2009;133:198-207.
48. Sweiss NJ, Salloum R, Ghandi S, Alegre ML, Sawaqed R, Badaracco M, Pursell K, Pitrak D, Baughman RP, Moller DR, Garcia JG, Niewold TB. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS One. 2010;5:e9088.
49. Hermaszewski RA, Webster AD. Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. Q J Med. 1993;86:31-42.
50. Chen H, Liao O, Holl TM, Snowden P, Ueda Y, Kelsoe G. Genetic regulation of pristine-induced oil granuloma responses. Int J Exp Pathol. 2010;91:472-483.

2.2

Granulomatous common variable immunodeficiency: the extent of disease

Kamphuis LS, Kwekkeboom DJ, Driesen GJ, van der Burg M, Atmaca S, Missotten T, van Laar JA, Dalm VA, van Zelm MC, van Hagen PM

Submitted for publication

Abstract

Granulomatous disease occurs in 10-22% of the patients with common variable immunodefiency (CVID). The presence of granulomas in CVID is associated with a worse prognosis and increased prevalence of lymphoproliferative disorders. This study was performed to investigate the frequency and to identify markers for granulomatous CVID.

Patients and methods

In this study we present the data of 37 CVID patients prospectively and randomly analyzed with an OctreoScan to screen for granulomatous lesions. Flow cytometric analyses involved peripheral blood lymphocytes and B cell subsets. Serum levels of the granulomatous markers angiotensin converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) were measured.

Results

The OctreoScan was negative in 11 patients, 5 patients had one lesions and 21 patients had more than one lesion. Most localizations were found in the lungs (73%), but salivary and inguinal glands lesions were common as well (respectively 12% and 19%). In 5 of the 26 patients with lesions it was histological proven to be granulomas. B cell subsets, T cell subsets and monocyte numbers do not differ significantly between non-granulomatous and granulomatous CVID. Serum levels of ACE were elevated in 8 of the 24 patients. Of these 8 patients, 7 patients had granulomas. Contradictionary to ACE, no correlation was found between granulomatous CVID and sIL-2R.

Conclusion

Our preliminary data suggest that based on OctreoScan the percentage of granulomatous disease in CVID is underestimated. Probably due to the fact that after diagnosing CVID no further research is performed to look if there are granulomas. 70% of the CVID patients in this study had lesions suspected for granulomas. Elevated levels of serum ACE may indicate granulomatous disease in CVID. Prospective studies are warranted to investigate the role of total body scintigraphy in patients with CVID.

Introduction

Common variable immunodefiency (CVID) is characterized by hypogammaglobulinaemia, recurrent infections and an impaired B cell compartment. A maturation arrest in the B cell development and/or disturbed B-T cell interaction results at the end in an antibody deficiency with poor vaccination responses (1). Granulomas can be found in a subgroup of patients with CVID. This subgroup is often regarded as having a "sarcoid-like syndrome" and are reported to have an impaired prognosis (2). In previous reports it has been estimated that in CVID, granulomatous disease occurs in 10-22% of the patients (3-5).

Sarcoidosis is a multisystem disorder with a hallmark of non-caseating granulomas (2, 6, 7). Recently we reported abundant perigranuloma B cell infiltration including IgA producing plasma cell involvement in sarcoid granulomas (8). In contrast to patients with CVID, sarcoidosis patients have normal serum immunoglobulin levels and normal antigen-specific immunoglobulin responses (7). Sarcoid granulomas can produce serological markers such as angiotensin-converting enzyme (ACE) and soluble interleukin-2 receptor (sIL-2R) (28, 29). ACE and sIL-2R are widely used in the assessment and follow-up of sarcoidosis (1, 5).

Recognition of granulomatous CVID is of great clinical importance. The presence of granulomas in CVID is associated with a worse prognosis because of organ damage and increased prevalence of lymphoproliferative disorders (8). As a consequence, granulomatous CVID patients should be monitored at the outpatient clinic more intensively than CVID patients without granulomas.

Previously, the visualization of granuloma localizations was reported in patients with sarcoidosis with scintigraphy using a radiolabeled somatostatin analog (OctreoScan) (10, 11). This analog binds to granulomas but is not specific for granulomas only. Somatostatin receptors (Sst) are expressed in specific inflammation like granulomas but are also expressed by Sst baring tumors. Imaging techniques such as OctreoScan are a sensitive technique to visualize granuloma localizations and to define the extent of disease (9).

It is not exactly known how many CVID patients have granulomatous involvement. In this study we retrospectively evaluated CVID patients for granulomas by OctreoScan. The extent of disease in patients with granulomatous CVID on OctreoScan was compared with the disease pattern of sarcoidosis. Granuloma biomarkers ACE and sIL-2R and peripheral B-T and monocytes numbers were related in patients with non-granulomatous CVID with presumed granulomatous CVID based positive OctreoScans.

Patients and methods

Patients

In this prospective study we analyzed 37 random CVID patients with OcteoScan from January 1997 to December 2012. We compared the pattern of suspected and confirmed granulomatous CVID patients with 175 patients with sarcoidosis who underwent OctreoScan in the same time span. For diagnostic purposes, we performed tissue biopsies in four patients with granulomatous CVID in order to rule out malignancy. We assume that OctreoScan positive sites in CVID patients resemble granulomatous inflammation, tissue biopsies for conformation are not permitted for ethical reasons.

In vivo scintigraphy; OctreoScan

The used scintigraphy technique has been described extensively elsewhere (10-12). Summarizing; In-111 pentetreotide is a [In-111 DTPA-D-Phe-] conjugate of octreotide, a long-acting somatostatin analog (OctreoScan®). The recommended administered activity is 222 MBq (6 mCi) in adults. The amount of pentetreotide injected is 10-20 mcg; that dose is not expected to have a clinically significant pharmacologic effect. Images are acquired at 4 and 24 hour and on indication or 48 hour post injection. The 48 hour images may be needed when there is significant bowel activity at 24 hour, which may potentially obscure lesions. We studied and compared retrospectively the extent of inflammatory sites in both patient groups.

Serum analysis

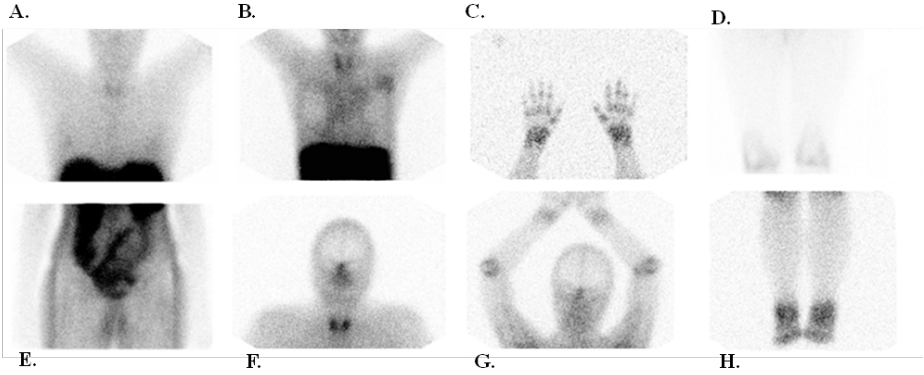
Serum levels of ACE were determined in 24 patients. Serum ACE levels was measured using the Bühlmann ACE kinetic test (Siemens Medical Solutions Diagnostics, Breda, The Netherlands). An enzyme-linked immunosorbent assay (ELISA, Diaclone, Besançon, France) was used to detect serum levels of sIL-2R in 28 patients. Serum ACE levels > 68 U/l and serum sIL-2R levels > 2500 pg/ml (reference values) was considered increased.

Flow cytometric analysis of peripheral blood lymphocytes and B cell subsets

Diagnostic work-up and detailed studies of blood were carried out with informed consent following the Declaration of Helsinki and according to the guidelines of the Medical Ethics Committee of Erasmus MC. Absolute counts of blood CD4+ and CD8+ T cells as well as CD16/56+ NK cells and CD19+ B cells were obtained with a diagnostic lyse-no-wash

protocol for 19 patients with granulomatous CVID. Furthermore, 8-color flow cytometric immunophenotyping was performed to detect transitional, naive mature and six memory B cell subsets using the following monoclonal antibodies: CD24-PB (SN3; ExBio), CD45-PO (HI30; Invitrogen), IgM-FITC, IgD-PE, IgG-PE, IgA-PE (all goat polyclonal from SBA), CD19-PerCP-Cy5.5(SJ25C1),CD21-PE-Cy7(B-ly4),CD27-APC(L128),CD38-APC-H7 (HB7), IgD-biotin (IA6-2; all from BD Biosciences). Biotinylated antibodies were detected with Streptavidin PE-Cy7 (eBioscience).

Figure 1. Examples of OctreoScan in CVID patients.



A, negative OctreoScan; B, uptake in the lungs and axillae; C, uptake in joints of the hand; D, uptake in the knees; E, cutaneous uptake; F, uptake in the nose; G, uptake in shoulder; H, uptake in ankles.

Results

Scintigraphy was negative in 11 of the 37 CVID patients (30%), 5 patients (14%) had one lesion suspected for granulomas and 21 patients (57%) had more than one lesion on OctreoScan. Figure 1 shows 2 examples of OctreoScan uptake in CVID patients. Similar to Resnick et al, most localizations of granulomatous CVID were found in the lungs (73%), but localizations in salivary and inguinal glands were common as well (respectively 12% and 19%) (13). In 4 of the 26 patients with uptake on OctreoScan a biopsy was taken for differential diagnostic purposes, which revealed non-necrotizing granulomas. We compared granuloma sites as detected with the OctreoScan in granulomatous CVID and a group of 175 sarcoidosis patients. Both groups showed almost a similar pattern. These results are summarized in Table 1.

Table 1. Lesions on OctreoScan in 37 patients with CVID and a group of 175 sarcoidosis patients in percentages (%).

Involved sites	CVID	Sarcoidosis
Thoracic	73	94
Hilar	31	71
Mediastinal	19	47
Lung parenchyma	38	38
Salivary glands	12	33
Inguinal	19	18
Skin	8	6

Serum ACE levels were measured in 24 CVID patients and in 8/24 these were elevated. Of these 8, 7 (88%), showed uptake on OctreoScan. In 24 of the 28 patients serum levels of sIL-2R were elevated. In contrast to ACE, no correlation was found between granulomatous CVID and sIL-2R. Mean serum sIL-2R level of patients with uptake on OctreoScan was 5574 pg/ml (range 5507 pg/ml - 19891 pg/ml) versus 8919 pg/ml (range 2998 pg/ml - 29793 pg/ml) of patients with no uptake.

In order to study if peripheral blood cell counts reflect the presence of granulomas we compared the results with the data of our CVID cohort. In 19 patients with SRS uptake the peripheral blood B- and T cell subsets were determined. Mononuclear cell numbers were extracted from leucocyte differential. The B cell subsets, T cell subsets and monocyte numbers do not differ significantly (Table 2).

Table 2. Peripheral blood lymphocyte levels of non-granulomatous and granulomatous CVID patients.

	Non-granulomatous CVID (n= 58)	Granulomatous CVID (n= 19)	P-value
T cells/μL	120	1610	0.06
Natural killers cells/μL	20	10	0.65
B cells/μL	30	10	0.57
Transitional B cells	12.9	6.7	0.79
Naive mature B cells	211.9	84.1	0.07
Natural effector B cells	65.6	25.4	0.29
CD27+IgM+	2.1	2.0	0.41
CD27+IgG+	7.6	2.7	0.45
CD27-IgG+	6.7	2.7	0.14
CD27+IgA+	4.4	2.2	0.24
CD27-IgA+	6.0	3.6	0.56
Plasma cells	0.2	0.2	0.91

Statistical analyses were performed using the Mann-Whitney test (SPSS v18.0).

Discussion

In this study, we analyzed the presence of granulomas in CVID patients. The results suggests that the frequency of granulomas is underestimated and that elevated levels of serum ACE may indicate granulomatous disease in CVID.

In the literature only a few reports on granulomatous CVID exist and reported frequency of granulomas is low (based on autopsy records) (4, 5). The results of our study suggest that, granulomatous lesions in CVID are more prevalent than expected from previous data. However, we have to take in regard that in only four out of 26 patients the lesions on scintigraphy were confirmed by histopathological examination. No other material was available for histopathological examination. OctreoScan has been performed for diagnostic work-up in patients with granulomatous disease in order to determine the affected organs. This technique is used to determine the extent of disease, for directed radiological examination and to explore a biopsy site (12, 30). A withdrawal of this technique is aspecific accumulation of radioactivity in the liver and specific visualization of the spleen which makes granuloma detection unreliable in these frequently affected organs. Since randomly biopsies were made, the presented data suggest an underestimation of granulomatous lesions in CVID patients.

The choice of scintigraphy may be a matter of debate. On 18F-FDG PET granulomatous lesions can be visualized as well, however as with OctreoScan, histopathological confirmation is sparse. No data are reported concerning the sensitivity between OctreoScan and 18F-FDG PET. In a preliminary study in our hospital 7 sarcoidosis patients underwent an OctreoScan and 18F-FDG PET. OctreoScan reveals more inflammatory localizations (data not shown).

We compared the affected organs between granulomatous CVID and sarcoidosis patients, showing no differences in affected sites. In previous studies and similar to our study, it has been reported that lungs were affected more frequently (4, 5).

Analysis of lymphocyte subsets in granulomatous CVID and non-granulomatous CVID did not reach significant differences suggesting that peripheral T-B lymphocytes and monocytes do not predict granuloma formation.

ACE and sIL-2R are widely used in the assessment of sarcoidosis (16). Increased serum levels of ACE are reported in 50% of the patients with sarcoidosis and is supposed to reflect the total granuloma mass (13, 14). This study showed elevated levels of serum ACE in 8 of the 24 CVID patients. Of the 8 patients, 7 patients had granulomas (88%, i.e. positive uptake on scintigraphy). Contradictionary to ACE, no correlation was found between granulomatous CVID and sIL-2R. Therefore elevated levels of serum ACE in CVID patients are suggestive for granulomas and could be a potential marker for granulomas in CVID patients.

Granulomatous lesions in CVID have a significant negative effect on survival, thus early detection is important in the management of the disease process (17). In summary the presented data suggest an underestimation of granulomas in CVID and implies to search for tools to improve predictive information on this subject. The consequences of our data warrants a prospective trial in which scintigraphy will be performed at diagnosis and certain time points with a long-term follow up in order to evaluate morbidity and mortality.

References

1. Möllers M, Aries SP, Drömann D, Mascher B, Braun J, Dalhoff K. Intracellular cytokine repertoire in different T cell subsets from patients with sarcoidosis. Thorax. 2001;56:497-493.
2. Vourlekis JS, Sawyer RT, Newman LS. Sarcoidosis: development in etiology, immunology, and therapeutics. Adv Intern Med. 2000;45:209-257.
3. Hillerdal G, Nou E, Osterman K. Sarcoidosis: epidemiology and prognosis. A 15-year European study. Am Rev Respir Dis. 1984;130:29.
4. Cunningham- Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1992;92:34.
5. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, Vlkova M, Hernandez M, Detkova D, Bos PR, Poerksen G, von Bernuth H, Baumann U, Goldacker S, Gutenberger S, Schlesier M, Bergeron-van der Cruyssen F, Le Garff M, DebréP, Jacobs R, Jones J, Bateman E, Litzman J, Hagen van PM, Plebani A, Schmidt RE, Thon V, Quinti I, Espanol T, Webster AD, Chapel H, Vihinen M, Oksenhendler E, Peter HH, Warnatz K. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111:77.
6. Rybickie BA, Major M, Popovich J Jr, Maliarik MJ, Iannuzzie MC. Racial differences in sarcoidosis incidence: a 5 year study in a health maintenance organization. Am J Epidemiol. 1997;145:234-241.
7. The joint statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG). Statement on sarcoidosis. Am J Respir Crit Care Med. 1999;160:736-755.
8. Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis?. Am J Respir Crit Care Med. 2013;187:406-416.
9. Izbicki G, Chavko R, Banauch GI, Weiden MD, Berger KI, Aldrich TK, Hall C, Kelly KJ, Prezant DJ. World Trade Center "sarcoid-like" granulomatous pulmonary disease in New York City Fire Department rescue workers. Chest. 2007;131:1414-1423.
10. Bokum ten AMC, Hofland LJ, Jong de G, Bouma J, Melief MJ, Kwekkeboom DJ, Schonbrunn A, Mooy ACM, Laman JD, Lamberts SWJ, Hagen van PM. Immunohistochemical localization of somatostatin receptor sst2A in sarcoid granulomas. European Journal of Clinical Investigation. 1999; 29:630–636.
11. Dalm V, Hagen van PM, Krenning EP. The role of octreotide scintigraphy in rheumatoid arthritis and sarcoidosis. Q J NUCL MED. 2003; 47:270-278.
12. Kwekkeboom DJ, Krenning EP, Kho GS, Breeman WA, van Hagen PM. Somatostatine receptor imaging in patients with sarcoidosis. Eur J Nucl Med. 1998;25:1284-1292.
13. Krenning EP, Bakker WH, Kooij PP, Breeman WA, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwekkeboom DJ. Somatostatine receptor scintigraphy with indium -111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry and comparison with iodine- 123- Tyr-3-octreotide. J Nucl Med. 1992;33:652-658.
14. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012;119:1650-1657.

15. Müller-Quernheim J. Serum markers for the staging of disease activity of sarcoidosis and other interstitial lung diseases of unknown etiology. Sarcoidosis Vasc Diffuse Lung Dis. 1998;15:22-37.
16. Bates CA, Ellison MC, Lynch DA, Cool CD, Brown KK, Routes JM. Granulomatous-lymphocytic lung disease shortens survival in common variable immunodeficiency. J Allergy Clin Immunol. 2004;114:415-421.
17. Sweiss NJ, Salloum R, Gandhi S, Alegre ML, Sawaqed R, Badaracco M, Pursell K, Pitrak D, Baughman RP, Moller DR, Garcia JG,Niewold TB. Significant CD4, CD8 an d CD 19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS One. 2010;5:e9088.
18. Hedfors E, Holm G, Pettersson D. Lymphocyte subpopulations in sarcoidosis. Clin Exp Immunol. 1974;17:219-226.
19. Kataria YP, Lobuglio AF, Bromberg PA, Hurtubise PE. Sarcoid lymphocytes: B- and T-cell quantitation. Ann N Y Acad Sci. 1976; 278:69-79.
20. Ross JJ, Katz JD. Cryptococcal meningitis and sarcoidosis. Scan J Infect Dis. 2002;34:937-939.
21. Cancelli I, Merlino G, Serafini A, Valente M, Gigli GL. Sarcoidosis as risk factor for cryptococcal meningitis in an apparently immunocompetent patient. Neurol Sci. 2008;29:33-35.
22. Riha RL, Allen RK Cryptococcosis and sarcoidosis: strange bed-fellows. A report of five cases. Sarcoidosis VascDiffuse Lung Dis. 2004; 21:71–76.
23. Warnatz K, Denz A, Dräger R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002;99:1544-1551.
24. Mert A, Bilir M, Ozaras R, Tabak F, Karayel T, Senturk H. Results of hepatitis B vaccination in sarcoidosis. Respiration. 2000;67:542-545.
25. Studdy PR, Bird R. Serum angiotensine converting enzyme in sarcoidosis—its value in present clinical practice. Ann Clin Biochem. 1989;26 :13.
26. Silverstein E, Friedland J, Lyons HA, Gourin A. Elevation of angiotensin-converting enzyme in granulomatous lymph nodes and serum sarcoidosis: clinical and possible pathogenic significance. Ann N Y Acad Sci. 1976;278:498-513.
27. Driessen GJ et al. B- cell replication history and somatic hypermutation status identify distinct pathophysiologic backgrounds in common variable immunodeficiency. Blood. 2011;118:6814-6823.
28. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
29. Bargali E, Bianchi N, Margollicci M, et al. Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. Scand J Clin Lan Invest. 2008;68:479-483.
30. Kamphuis LS, Kwekkeboom DJ, Missotten TO, Baarsma GS, Dalm VA, Dik WA, Timmermans WM, van Daele PL, van Hagen PM, van Laar JA. Somatostatin receptor scintigraphy patterns in patients with sarcoidosis. Clin Nucl Med. 2015;Oct 21.

2.3

Prevalence of atopic disease in patients with sarcoidosis

Hajdarbegovic E, Kamphuis L, van Laar J, van Hagen M, Nijsten T, Thio B

Allergy Asthma Proc. 2014;35:57-61

Abstract

It remains unclear whether atopy is associated with the occurrence of sarcoidosis or affects its severity. The purpose of this study was to compare the lifetime prevalence of atopic eczema, asthma, and hay fever in sarcoidosis patients with controls and to assess whether atopy influences the severity of sarcoidosis.

Methods

The prevalence of atopic disorders assessed with a validated postal questionnaire in sarcoidosis patients with pulmonary, uveitis, and cutaneous sarcoidosis was compared with that of their domestic partners in a case-control study. The serological parameters, the pulmonary function tests, and the high-resolution computed tomography (HRCT) scans of atopic and nonatopic sarcoidosis patients were compared in a nested cohort. Multivariate logistic regression models were used to calculated the odd ratios (ORs) and the 95% confidence intervals (CIs).

Results

Two hundred twente-five sarcoidosis patients and 177 controls were included. The prevalences of atopic eczema, asthma, and hay fever were comparable between patients and controls (12.4% versus 12.4%, 5.3% versus 5.6%, and 16.9% versus 15.8%, respectively). After adjusting for gender and ethnicity, those with sarcoidosis and a history of atopic eczema were significantly less likely to have uveitis (OR, 0.30; 95% CI, 0.13-0.71). Within the sarcoidosis cohort, the distributions of serological markers, the lung function tests, and the HRCT scans were similar between atopic and nonatopic patients. Atopy is not associated with the occurrence of sarcoidosis, but atopic eczema may decrease the likelihood of eye involvement.

Background

Sarcoidosis is a systemic granulomatous disease of variable clinical presentation and course. Over 90% of the patients have thoracic involvement, but the skin, the eyes and other organs may also be affected (1). The annual incidence is estimated at 20 per 100,000 (2). It is thought to be mainly T-helper type 1 (Th-1) driven, but the exact etiology is unknown. Predisposing factors for sarcoidosis are black race, female sex, age between 20 and 40 years and a family history of sarcoidosis (3). An uncontrolled study in 41 Turkish sarcoidosis patients revealed a lower prevalence of asthma and positive skin prick tests as compared with the expected rate based on national data (4).

Two studies by Hattori et al, reported lower total serum immunoglobulin E (IgE) in sarcoidosis patients and fewer parenchymal lung lesions in chest X-rays in atopic sarcoidosis patients (19% vs. 37%; p=0.018) (5, 6). However, no evidence of a lower prevalence of atopic asthma was found in a cross-sectional survey among 136 sarcoidosis patients (7). Therefore, it remains unclear whether the prevalence of atopic disorders is decreased in sarcoidosis patients and whether atopy affects the severity of sarcoidosis. In this case-control study, the association between atopic disorders, sarcoidosis and detailed sarcoidosis characteristics in the largest cohort to date was investigated.

Patients and methods

Study population

We approached by 371 consecutive patients with sarcoidosis diagnosed between 2000 and 2012 at the Clinical Immunology outpatient clinic at the Erasmus Medical Center. Our center is a tertiary referral center with expertise in sarcoidal uveitis and cutaneous sarcoidosis. Patients' medical records were available to the study team. The spouses/partners of the contacted patients were asked to act as controls. Participants were considered to be of Dutch ancestry if both of their parents were born in the Netherlands. Caribbean and Suriname subjects were those in whom one of the parents was born either in Suriname or the Caribbean. This study was conducted in accordance with the Declaration of Helsinki and the medical ethical committee of the Erasmus Medical Center provided a waiver for this single-subject questionnaire-based study.

Patient-reported atopic features

Patients were sent an invitation letter, the questionnaire and a prepaid return envelope. Non-responders were contacted a second time via mail. The definitions of the atopic features (i.e. atopic eczema, hay fever and asthma) were self-reported and based on items from the European Community Respiratory Health Survey and International Study of Asthma and Allergies in Children protocol (8, 9). Subjects were considered to have had asthma if they answered the question "Have you ever been diagnosed with asthma by a doctor?" positively and if the answer to the question "How old were you when you were diagnosed with asthma?" was younger than 25 years. The criteria for the diagnosis of atopic dermatitis were: atopic dermatitis diagnosed by a physician, before the age of 25 and a duration of more than 1 year. For diagnosis of hay fever patients had to answer the next question affirmatively; "Have you ever had hay fever before the age of 25 years?". The age of 25 years was used as cut-off in order to increase the specificity as atopic disorders mainly have their onset during childhood and adolescence. This prevents misclassification of other forms of eczema as atopic eczema and of chronic obstructive pulmonary disease as atopic asthma. Patients were also asked about the use of topical corticosteroids for their atopic dermatitis, inhalants for the asthma and nasal sprays or anti-histaminics for the hay fever. The presence of atopic features in the first degree relatives was also assessed through this questionnaire.

Sarcoidosis types, activity and severity

Sarcoidosis patients were further classified into non-exclusive three groups: pulmonary sarcoidosis, sarcoidal uveitis and cutaneous sarcoidosis. The diagnosis of sarcoidosis was established according to the guidelines of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders statement on sarcoidosis (10). The diagnosis of (posterior or anterior) uveitis was made by certified immunologists and confirmed by ophthalmologic examination. The diagnosis of cutaneous sarcoidosis was confirmed by biopsy.

To assess the disease activity and severity (i.e., organ involvement and serological markers) radiological, serological and pulmonary function tests were performed on clinical indication. Serological tests included erythrocyte sedimentation rate, T-lymphocyte levels, serum ACE and serum calcium. All patients suspected of having sarcoidosis routinely undergo high

resolution computed tomography scanning at our center. Modified Scadding scoring system was applied to High resolution CT-scanning (HRCT) in order to assess pulmonary sarcoidosis (11). All HRCTs were graded according to the Scadding system by a certified radiologist (12). The Scadding scale: 0 re presents a normal chest HRCT, stage 1: bilateral hilar lymphadenopathy, stage 2: stage 1+ pulmonary infiltrates, stage 3: there are pulmonary infiltrates without hilar lymphadenopathy and stage 4 shows pulmonary fibrosis.

In pulmonary sarcoidosis, pulmonary functioning may be impaired even in the absence of radiological abnormalities. Transfer factor for carbon monoxide (TLCO) and total lung capacity (TLC) are characteristically affected but the forced vital capacity (FVC) and forced expiratory 1 second volume (FEV1) are also frequently decreased (13,14). Pulmonary function tests were performed according to the local protocol at the Pulmonology Department. The values of the parameters were expressed as a percentage of the expected value corrected for ethnicity, sex, age and haemoglobin levels. The classic serological parameters of the disease activity are angiotensin-converting enzyme (ACE), calcium (CA) and T-cell numbers. These were performed at the time of diagnosis according to the protocol of the Medical Laboratory at the Erasmus Medical Center.

Patients were also asked to report the name of their current medication for sarcoidosis. If no medication was currently used and patients declared so the sarcoidosis was considered resolved.

Statistics

For sample size calculations, we assumed a prevalence of 20% of atopic disorders in our control group, as previously found (15) and expected half of this proportion in sarcoidosis patients. A convenience sample of about 200 cases was deemed resonable. We compared the prevalence of atopic features between the patients and the controls. In the cohort of sarcoidosis patients we also investigated whether the presence of atopic features was related to specific organ involvement in sarcoidosis (i.e. pulmonary, uveitis and cutaneous sarcoidosis).

The Chi-squared test was used to test for statistical differences in proportions and Mann Whitney-U tests for non-parametric testing. Age, sex and ancestry were considered confounders in the relation between sarcoidosis and atopy. Multivariate logistic regression models were used to calculate adjusted odds ratios (OR) and 95% confidence intervals (95% CI). Variables with $p<0.20$ in the univariate analysis were included in multivariate models. Values of $p<0.05$ were considered significant. All data were analyzed using IBM SPSS version 19.0 (Chicago, Illinois, 2011).

Results

Subject characteristics

Of the 371 patients, 225 (61%) replied to our mailing with 177 control partners. The median age was 50 years for patients and 52 for controls. The controls were significantly more often males (54% vs. 39% p=0.003) and of Dutch ancestry (79% vs. 69% p=0.022) as compared with patients (Table 1).

Table 1. Subject characteristics.

	Sarcoidosis Patients n = 225	Controls n = 177	p Value
Median age, yr (IQR)	50 (41-61)	52 (40-63)	0.928
Male sex	88 (39%)	96 (54%)	0.003
Family history of atopy	127 (56%)	99 (56%)	0.918
Ancestry			
Dutch	152 (69%)	137 (79%)	0.022
Caribbean and Suriname	43 (19%)	18 (10%)	0.013
Other	30 (13%)	22 (13%)	0.796
Pulmonary sarcoidosis	166 (74%)		
Scadding score*			
Stage 0	26 (16%)		
Stage 1	45 (27%)		
Stage 2	59 (36%)		
Stage 3	9 (5%)		
Stage 4	18 (11%)		
no HRCT	9 (5%)		
Sarcoidosis uveitis	126 (60%)	n/a	
Cutaneous sarcoidosis	92(45%)	n/a	
Median duration of sarcoidosis, yr	5.0 (IQR, 2.0-10.0)	n/a	

*0, normal chest HRCT; 1, bilateral hilar lymphadenopathy; 2, stage 1+ pulmonary infiltrates; 3, pulmonary infiltrates without hilar lymphadenopathy; 4, pulmonary fibrosis. IQR; interquartile range; HRCT = high-resolution computed tomography; n/a = not applicable.

Almost twice as many patients were of Surinam or Caribbean descent as compared with their partners (19% vs. 10% p=0.013).

Pulmonary involvement was found in 166 (74%) and in 157(95%) of these patients HRCT-scanning was performed. Uveitis and cutaneous sarcoidosis were seen in 126 (60%) and 92 (45%) of patients respectively.

Atopic features and sarcoidosis

Sarcoidosis patients with pulmonary sarcoidosis did not differ from their partners regarding frequency of self-reported diagnoses of asthma, hay fever or eczema (Table 2). Moreover, drug use for atopic disorders and proportions of positive family histories did not differ across patients and controls. The same was the case for patients with cutaneous sarcoidosis. Adjusting for the confounders did not alter these results. However, patients with sarcoidosis uveitis reported eczema less often than the controls (26% vs. 12%, p=0.045) (Table 2).

Table 2. Prevalence of self-reported atopic disorders and their medication use.

	Sarcoidosis n = 225	Controls n = 77	p Value
Atopic eczema			
Ever	28 (12.4%)	22 (12.4%)	0.996
Current topical corticosteroid use	39 (18.6%)	21 (12.0%)	0.124
Family history	70 (30.1%)	50 (28.2%)	0.534
Atopic asthma			
Ever	12 (5.3%)	10 (5.6%)	0.890
Current use of inhalators	6 (2.7%)	5 (2.8%)	0.923
Family history	56 (25.0%)	43 (24.3%)	0.891
Hay fever			
Ever	38 (16.9%)	18 (15.8%)	0.774
Current use of nasal spray / antihistaminics	37 (17.6%)	26 (14.7%)	0.437
Family history	89 (39.6%)	67 (37.9%)	0.725
Any atopic disorder	60 (26.7%)	44 (24.9%)	0.681

This association yielded an unadjusted OR 0.32 95%CI[0.14-0.73] and after adjusting for age, ancestry and sex this was OR 0.30 95%CI[0.13-0.71] (Table 3).

Table 3. Uni- and multivariate logistic analyses of the risk of sarcoidal uveitis.

n = 402	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Eczema	0.32 (0.14-0.73)	0.30 (0.13-0.71)
Age, yr (per 10 yr)	1.12 (0.95-1.31)	1.16 (0.98-1.37)
Dutch ancestry	0.50 (0.31-0.79)	0.50 (0.31-0.81)
Male sex	0.42 (0.27-0.66)	0.42 (0.27-0.67)

Adjusted for all the variables in table 3.
CI = confidence interval; OR = odds ratio.

Sarcoidosis in atopic patients

We also analyzed the differences in the activity and the severity of sarcoidosis in atopic and non-atopic patients. No statistically significant differences were found in the distribution of serum ACE, calcium, erythrocyte sedimentation rate, T-cell numbers and lung function tests (TLC, FVC, FEV1 and TLCO; Table 4). The HCRT modified Scadding scores were comparable between atopic and non-atopic sarcoidosis patients. A history of atopic diseases did not affect resolution rates (15% vs. 20%, p=0.895), TNF-alpha blocker use (20% vs. 21%, p=0.8990 and methotrexate use (20% vs. 30%, p=0.340) among patients with sarcoidosis.

Table 4. Pulmonary sarcoidosis activity and severity in atopic vs nonatopic patients at the time of diagnosis sarcoidosis.

	Any Atopy n = 43	No Atopy n = 123	p Value
Serum ACE, ug/L	52 (IQR, 26-112)	47 (IQR, 30-86)	0.325
Serum CA, mmol/L	2.35 (IQR, 2.29-2.43)	2.40 (IQR, 2.33-2.47)	0.211
Erythrocyte sedimentation rate, mm/hr	12 (IQR 6.0-24)	15 (IQR, 6.0-31)	0.958
T-cells, * 109/L	0.75 (0.41-1.02)	0.73 (IQR, 0.53-1.22)	1.000
Total lung capacity, %ref	86 (IQR, 68-97)	79 (IQR, 17-90)	0.297
Forced vital capacity, %ref	96 (IQR, 72-103)	100 (IQR, 89-112)	0.247
Forced expiratory volume in 1 s, %ref	86 (IQR, 64-99)	92 (IQR, 76-105)	0.938
Transfer factor for carbon monoxide/ diffusion capacity for carbon monoxide, %ref	74 (IQR, 52-91)	72 (IQR, 52-82)	0.938
Sarcoidosis Scadding stage HRCT	n = 39	n = 118	
0	8 (21%)	18 (15%)	
1	7 (18%)	38 (33%)	
2	15 (38%)	44 (37%)	
3	4 (10%)	5 (4%)	
4	5 (13%)	13 (11%)	0.341*

* The p value for trend.

IQR = interquartile range; HRCT = high-resolution computed tomography; ACE = angiotensin-converting enzyme; %ref = expressed as percentage of reference group.

Discussion

No association between atopy and the development of sarcoidosis was found in this study. Having an atopic disorder was also not associated with attenuated disease. However, atopic eczema is associated with a lower occurence of sarcoidal uveitis. This strong negative association remained after adjusting for confounders.

Our study also confirmed the classic risk factors (black race and female sex) for sarcoidosis. Sarcoidosis is currently perceived as a Th1-mediated disease, whereas atopic disorders are categorized as exclusively Th2 conditions. Antagonism between Th1 and Th2 axes has been the subject of previous studies and atopy has been linked to a lower prevalence and a less severe course of auto-immune diseases like diabetes mellitus type-1, rheumatoid arthritis and multiple sclerosis (16). The ACCESS (A Case Controlled Etiologis Study of Sarcoidosis) study also revealed that sensitization to household animal dust and feathers conferred a lower risk for the development of sarcoidosis (17).

The Turkish and the Japanese studies reported a lower prevalence of positive tests for atopy, including skin prick tests and total serum IgE levels in subjects with sarcoidosis (4, 5). In contrast, another questionnaire based study performed in New Zealand did not confirm these findings (18). Questionnaire-based studies may be more appropriate in this setting as atopic disorders, especially atopic eczema, are generally more active during childhood. Moreover, negative skin prick tests and lower total serum IgE levels may reflect an anergy of sarcoidosis instead of an absence of atopy. Anergy in sarcoidosis is a well-recognized phenomenon. Nevertheless, no single impairment has been pinpointed to be the cause. The anergy of sarcoidosis has been ascribed to the generally increased numbers of Tregs (19) and inert dendritic cells (20). Clinically, it results in reduced efficacy of vaccination (21) and suppression of cell mediated hypersensitivity (22-24). It is plausible that this anergy affects the expression of atopy or reactivity to in vivo and in vitro atopy tests.

Our results showed that atopic eczema was negatively associated with uveitis. How eczematous skin may contribute in the protection of physically and immunologically isolated eyes is not clear from this study. This association was independent of traditional risk factors gender and ancestry which suggests a non-genetic effect. However this unexpected finding warrants replication in other studies.

The major weakness of our data are the dependence of self-reporting and retrospective assessment, with inherent recall bias. This is due to the nature of atopic disorders which mostly tend to wane in the adulthood. Recall bias could have played a role in the reporting of atopy. In addition, we have reported a cohort of patients referred to our university clinic with a relatively high incidence of uveitis which could also bias our results.

This is the first controlled study assessing the history of atopy. This is also the first study of sarcoidosis and atopy to take into account the three major types of sarcoidosis patients. The prevalence of atopic features in our control group is in concordance with prevalence found in a previous study (15). In future studies we recommend further investigation into the association of sarcoidosis uveitis and atopic eczema and if confirmed, investigation of the possible mechanisms.

References

1 Morgenthau AS, Iannuzzi MC. Recent advances in sarcoidosis. Chest. 2011; 139: 174-82.
2 Thomas KW, Hunninghake GW. Sarcoidosis. JAMA. 2003; 289: 3300-3.
3 Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003; 361: 1111-8.
4 Kokturk N, Han ER, Turktas H. Atopic status in patients with sarcoidosis. Allergy Asthma Proc. 2005; 26: 121-4.
5 Hattori T, Konno S, Takahashi A, et al. The role of atopy in the clinical course of pulmonary sarcoidosis in the Japanese population. Allergy Asthma Proc. 2010; 31: 238-43.
6 Hattori T, Konno S, Shigemura M, et al. Total serum IgE levels and atopic status in patients with sarcoidosis. Allergy Asthma Proc. 33: 90-4.
7 Wilsher M, Hopkins R, Zeng I, et al. Prevalence of asthma and atopy in sarcoidosis. Respirology (Carlton, Vic. 17: 285-90.
8 Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J. 1995; 8: 483-91.
9 Burney PG, Luczynska C, Chinn S, et al. The European Community Respiratory Health Survey. Eur Respir J. 1994; 7: 954-60.
10 Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med. 1999; 160: 736-55.
11 Remy-Jardin M, Giraud F, Remy J, et al. Pulmonary sarcoidosis: role of CT in the evaluation of disease activity and functional impairment and in prognosis assessment. Radiology. 1994; 191: 675-80.
12 Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. Br Med J. 1961; 2: 1165-72.
13 Alhamad EH, Lynch JP, 3rd, Martinez FJ. Pulmonary function tests in interstitial lung disease: what role do they have? Clin Chest Med. 2001; 22: 715-50, ix.
14 Sharma OP, Johnson R. Airway obstruction in sarcoidosis. A study of 123 nonsmoking black American patients with sarcoidosis. Chest. 1988; 94: 343-6.
15 Hajdarbegovic E, Nijsten T, Westgeest A, et al. Decreased prevalence of atopic features in patients with psoriatic arthritis, but not in psoriasis vulgaris. J Am Acad Dermatol. 2012.
16 Rabin RL, Levinson AI. The nexus between atopic disease and autoimmunity: a review of the epidemiological and mechanistic literature. Clin Exp Immunol. 2008; 153: 19-30.
17 Baughman RP, Teirstein AS, Judson MA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001; 164: 1885-9.
18 Wilsher M, Hopkins R, Zeng I, et al. Prevalence of asthma and atopy in sarcoidosis. Respirology. 2012; 17: 285-90.
19 Miyara M, Amoura Z, Parizot C, et al. The immune paradox of sarcoidosis and regulatory T cells. J Exp Med. 2006; 203: 359-70.
20 Mathew S, Bauer KL, Fischoeder A, et al. The anergic state in sarcoidosis is associated with diminished dendritic cell function. J Immunol. 2008; 181: 746-55.
21 Mert A, Bilir M, Ozaras R, et al. Results of hepatitis B vaccination in sarcoidosis. Respiration. 2000; 67: 543-5.
22 Morell F, Levy G, Orriols R, et al. Delayed cutaneous hypersensitivity tests and lymphopenia as activity markers in sarcoidosis. Chest. 2002; 121: 1239-44.

23 Demirkok SS, Basaranoglu M, Coker E, et al. Seasonality of the onset of symptoms, tuberculin test anergy and Kveim positive reaction in a large cohort of patients with sarcoidosis. Respirology. 2007; 12: 591-3.
24 Gupta D, Chetty M, Kumar N, et al. Anergy to tuberculin in sarcoidosis is not influenced by high prevalence of tuberculin sensitivity in the population. Sarcoidosis Vasc Diffuse Lung Dis. 2003; 20: 40-5.

Chapter 3

Diagnostics

Somatostatin receptor scintigraphy patterns in patients with sarcoidosis

Kamphuis LS, Kwekkeboom DJ, Missotten TO, Baarsma GS, Dalm VA, Dik WA, Timmermans WM, van Daele PL, van Hagen PM, van Laar JA

Clin Nucl Med. 2015;40:925-929

Abstract

Purpose

Sarcoidosis is a multisystem granulomatous disorder, most frequently involving the lungs, skin, or eyes. Somatostatin receptor scintigraphy (SRS) can visualize sarcoid granulomas through binding of a radionuclide-coupled somatostatin analog to somatostatin receptors that are expressed in sarcoidosis. Uptake and patterns on SRS were studied and correlated to clinical and conventional findings.

Methods

Data of 218 SRSs undertaken for the analysis of potential sarcoidosis were studied. These scintigraphies were retrospectively studied on intensity uptake degrees and localization of sarcoidosis-associated lesions, and compared with conventional radiological techniques (chest x-ray and CT).

Results

In all but 1 of the 175 evaluable patients, SRS demonstrated uptake. In patients with thoracic sarcoidosis-associated lesions, SRS improved the yield of visualization of chest x-ray in 20 (36%) and CT in 7 (32%) of histologically unproven patients, and in 31 (30%) and 8 (14%) of the histologically proven patients, respectively. Mediastinal lesions together with either eye, salivary glands, clavicular, or hilar localizations were most frequent demonstrated on SRS and constituted characteristic patterns. Exclusive extrapulmonary disease was found in 6% of the patients.

Conclusions

Somatostatin receptor scintigraphy enhances the yield of investigations in sarcoidosis patients and therefore provides a useful and sensitive imaging technique to monitor organ involvement and therapeutic efficacy in patients with sarcoidosis.

Introduction

Sarcoidosis is a multisystem disease of unknown origin and is located in > 90% intrathoracal (1). Traditionally, the diagnosis is based on compatible clinical and radiological findings (X-ray and CT-scan), the presence of non-caseating granulomas and exclusion of similarly presenting disorders (2). The yield of conventional imaging techniques can be augmented by novel radionuclear imaging.

Somatostatin receptor subtype 2 (sst2) is highly expressed in sarcoid granulomas and used as a substrate for somatostatin receptor scintigraphy (SRS) with 111In-DTPA-D-Phe1-octreotide, and the pendant in PET-imaging with 68Gallium labelled somatostatin analogues (3-6). 111In-DTPA-D-Phe1-octreotide shows a high affinity for the sst2 receptor and can therefore be used in the imaging of sarcoidosis (7).

Previous studies with SRS showed improved imaging in sarcoidosis (8, 9). So far, it is however scarcely studied in what percentage of patients the yield of clinical evaluations can be extended by SRS. The aim of this retrospective study was to evaluate the additive value in the clinical evaluation and determination of sarcoidosis by SRS.

Material and methods

Patients

This retrospective study included 218 patients of the Clinical Immunology outpatient clinic in the Erasmus University Medical Center that had SRS because of a clinical suspicion or follow up for sarcoidosis between June 1991 and June 2010 (Table 1).

Sarcoidosis was diagnosed according to the guidelines of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders statement on sarcoidosis (10). Exclusion criteria were a diagnosis other than sarcoidosis, steroid therapy during SRS or resolved disease (Figure 1).

Table 1. Patient characteristics of 175 patients with sarcoidosis.

Age in years (SD)	52 (13)
Disease duration in years (range)	2.6 (0-35)
Sex	
Female	101
Male	74
Race	
White	119
Black	56
Histological proof for sarcoidosis	
Yes	109
No	66
Negative SRS	1
1 localization on SRS	10
More than 1 localization on SRS	164

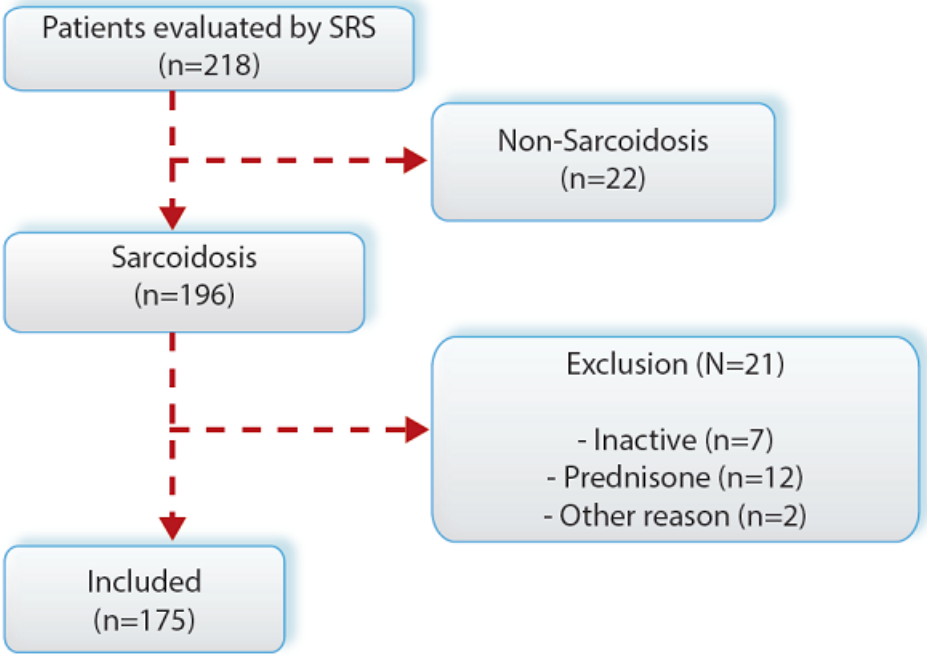
Somatostatin receptor scintigraphy

The scintigrams of all 218 patients were initially examined by nuclear medicine physicians and for this study independently re-examined by an investigator (L.S.K.) with supervision of a senior nuclear medicine physician (D.J.K.), without knowledge of the medical history or outcome of other investigations. Uptake of radioactivity in disease-related areas was graded on a four-point scale: 0, no uptake; 1, intensity less than that of the liver; 2, intensity identical to that of the liver; 3, intensity greater than that of the liver (8). Disease activity was scored for the following localizations: mediastinum, hila, lung parenchyma, extra pulmonary lymph nodes, central nervous system, eye, nose, salivary glands, oral mucosa, heart, skin, muscles and joints.

Conventional imaging

Conventional imaging of the chest (X-ray or CT-scan) was available for 158 patients. Chest X-rays (CXR) results were classified according the Scadding system (11, 12). The time interval between SRS and conventional imaging was less than 3 months and in a similar clinical situation, without change in clinical course or intervention with therapeutic agents (13,14).

Figure 1. Inclusion algorithm.



Schematic view of 218 included patients undergoing a SRS in the event of evaluation for sarcoidosis. Diagnosis of the 22 non-sarcoidosis patients included SLE (1), MS (1), idiopathic uveitis without signs of extra ocular disease (1), tuberculosis (1), fibromyalgia (1), idiopathic small fibre neuropathy (1), medullary thyroid carcinoma (1), granulomatous tattoo reaction (1), unspecified granulomatous dermatitis (1), Crohn's disease (1), granulomatous vasculitis surrounding sural nerve (presumably local eosinophilic granulomatosis with polyangiitis 1), inflammatory orbital process (1), carcinoid (1), idiopathic erythema nodosum (2), Sjögren's disease (1), polymyalgia rheumatica (1), gout (1), rheumatoid arthritis (1) or none (3).

Statistics

Statistical analyses were performed using the one-way ANOVA test and $\chi 2$ test for categorical data (SPSS version 18.0). A P-value < 0.05 was considered statistically significant.

Results

Patients

Out of the 218 patients analysed by SRS, 175 patients were included (Figure 1). Patient characteristics are summarized in Table 1. Granulomas could be demonstrated in 109 patients. The details for exclusion of the 43 patients are given in Figure 1. Of the 12 patients on prednisone 6 still demonstrated SRS uptake.

Localizations and typical patterns of distribution of radioactivity on SRS

Ten patients had one localization and 165 patients had more than one localization on SRS (Table 1). Figures 2 and 3 demonstrate examples of positive and negative SRS and the frequency of involved sites are summarized in Table 2. Table 3 shows significant patterns in sarcoidosis.

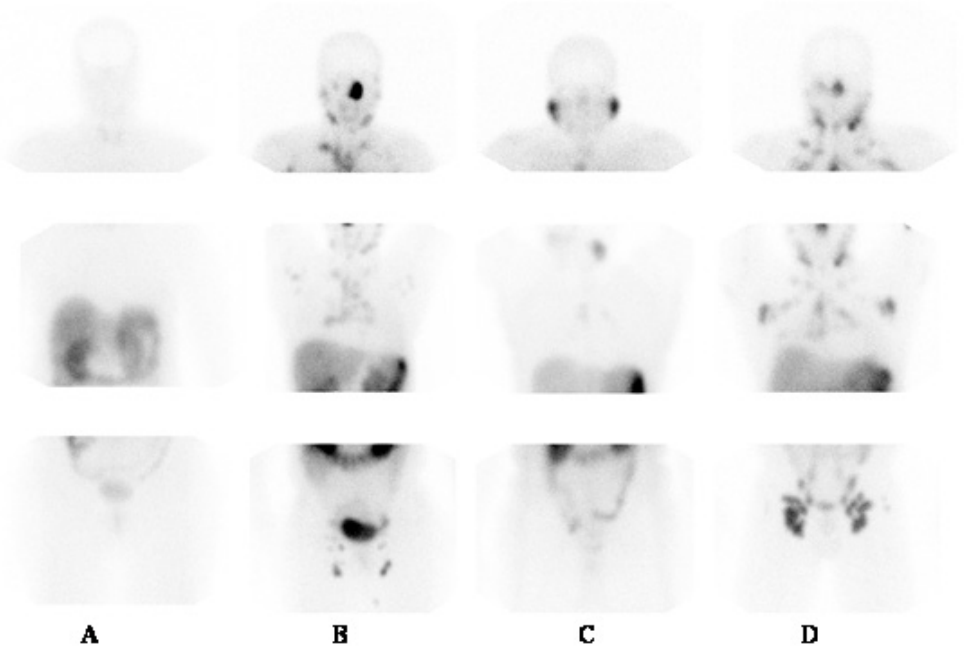
Table 2. Sarcoidosis involvement in 175 patients on SRS.

Involved Sites	n (%)
Thoracic	165 (94)
Hilar	118 (71)
Mediastinal	78 (47)
Lung parenchyma	63 (38)
Central nervous system	11 (6)
Eye	39 (22)
Nose	44 (25)
Salivary glands	58 (33)
Oral mucosa	3 (2)
Axillae	20 (11)
Clavicular	14 (8)
Heart	7 (4)
Inguinal	32 (18)
Skin	11 (6)
Muscle	1 (1)
Joints	17 (10)

Localization of SRS uptake in sarcoidosis patients evaluated with SRS.
Italic value is important to make clearly differentiate between all involved pulmonal sites and a subdivision of pulmonal sites.

SRS can enhance the yield of conventional imaging techniques
In both histologically proven and unproven sarcoidosis (109 and 66, respectively) all but one SRS demonstrated uptake. In the negative scan, granulomas were histologically demonstrated in hepatic portal lymph nodes where normal hepatic SRS uptake influences differentiation. In this case the initial radio nuclear medicine physician did report probable uptake probably in portal lymph nodes. This could initially not be visualized by ultrasonography, but later was confirmed by CT. The blinded researcher (L.S.K.) did not detect SRS uptake, hence the SRS was scored 0.

Figure 2. Examples of planar images of SRS.



A; no pathological uptake. B; pathological uptake is present in nose, lacrimal, parotid and submandibular glands, neck, mediastinum, hila, axillae, inguinal lymph nodes and skin. C; pathological uptake is present bilateral in the parotid glands and in mediastinum. D; nose, lacrimal glands, parotid and submandiblar glands with pathological uptake with bilateral pathological uptake in the supraclavicular, axillae, mediastinal and inguinal lymph nodes. In all images physiological bowel contamination and urinary bladder activity are seen in the abdomen.

SRS is superior in imaging than conventional imaging in thoracic sarcoidosis

In the histologically unproven patients, the combination of laboratory and/or bronchial fluid findings, typical patterns on radiological imaging combined to the SRS results established the diagnosis of sarcoidosis. Comparison of the SRS with conventional radiological imaging of the thoracic region showed that SRS augmented the yield. Respectively 56 and 22 CXR or CT could be compared with SRS. SRS improved the yield in 20 (36%) and 7 (32%) of those patients, respectively. CT did not add more positive results to the CXR (data not shown).

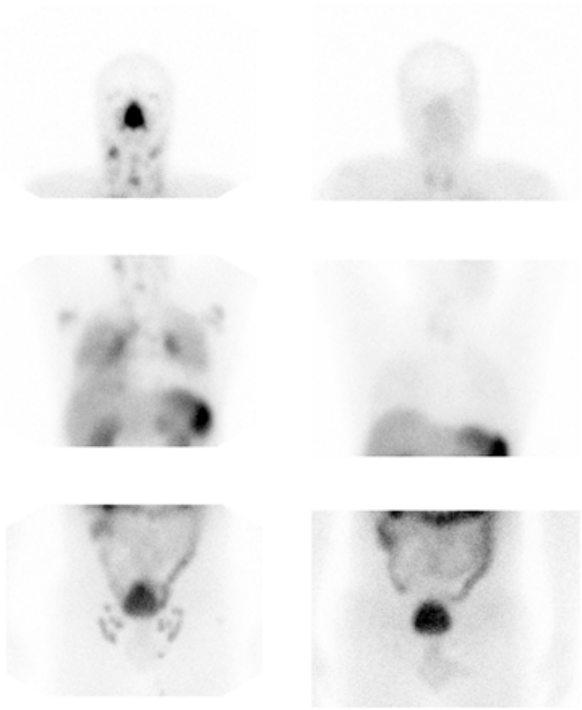
In the histological proven group respectively 102 and 59 patients had concomitant SRS and CXR or SRS and chest CT. There were no negative SRS in this group. SRS improved the visualization of thoracic localization in 31 (30%) and 8 (14%) patients, respectively (Table 4). Apart from granuloma localization, Figure 3 demonstrates a correlation of clinical activity of sarcoidosis with therapeutic efficacy.

Table 4. SRS compared to conventional imaging in patients with histologically proven sarcoidosis.

SRS-Conv	n = 109		SRS +	SRS-
		Conv +	102	1
		Conv -	6	0
SRS-CXR	n = 102		SRS +	SRS -
		CXR +	71	0
		CXR -	31	0
SRS-CT	n = 59		SRS +	SRS -
		CT +	51	0
		CT -	8	0
CT-CXR	n = 59		CXR +	CXR -
		CT +	42	8
		CT -	0	9

In 109 histological proven sarcoidosis patients conventional (Conv) techniques were compared in the establishment of the diagnosis. By use of SRS scans thoracic localizations were found in 102 patients and compared to either chest X-ray (CXR) or CT of the chest (CT).

Figure 3. SRS prior and after treatment with corticosteroids.



This patient, a woman of 37 years, was treated with corticosteroids for a period of 16 months and showed a good clinical response of this treatment on SRS. Her signs of shortness of breath and fatigue disappeared. Pathological uptake in the first image is present in nose, lacrimal, parotid and submandibular glands, neck, mediastinum, hila, lung parenchyma, axillae and inguinal lymph nodes.

Fibrosis

52 patients had lung parenchyma uptake on conventional imaging and 77 patients on SRS. Of the patients that were SRS positive, but negative on conventional thoracic imaging techniques, 5 developed pulmonary fibrosis shown on CT-scan ranging 3 - 8 years after SRS.

Table 3. Typical patterns of sarcoidosis on SRS, combinations, their prevalence, and significance.

	Thoracic	Hilar	Medi astinal	Lung parenchyma	Eye	Nose	Salivary glands	Axillae	Clavicular	Inguinal	Skin
Thoracic					43/165†	42/165	62/165†	22/165*	16/165*	36/165†	10/165
Hilar			63/118‡	36/118	29/118/*	3/118*	45/118	16/118	13/118†	29/118†	8/118
Mediastinal		62/78†		28/78	27/78‡	20/78	34/78†	10/78	14/78‡	18/78	6/78
Lung parenchyma		31/63	25/63		17/63	16/63	25/63	9/63	7/63	15/63	3/63
Eye	37/39†	28/39*	22/39†	16/39		15/39†	20/39‡	3/39	3/39	10/39	3/39
Nose	38/44	31/44*	20/44	20/44	16/44†		28/44‡	11/44‡	4/44	17/44‡	7/44‡
Salivary glands	52/58†	39/58	27/58*	24/58	20/58†	27/58†		17/58‡	5/58	20/58‡	6/58‡
Axillae	19/20*	14/20	10/20	13/20‡	4/20	11/20‡	17/20‡		2/20	14/20‡	6/20‡
Clavicular	14/14*	13/14‡	12/14‡	7/14	3/14	4/14	6/14	2/14		2/14	2/14
Inguinal	30/32†	25/32	16/32	16/32*	11/32*	17/32‡	20/32‡	14/32‡	2/32		6/32‡
Skin	10/11	9/11	6/11	4/11	4/11	9/11‡	9/11‡	7/11‡	1/11	8/11‡	

Statistical analyses were performed using x2 tests. For example, mediastinal lesions together with eye uptake demonstrated a significant characteristic pattern ($P < 0.001$), and pathological uptake in skin together with inguinal uptake is $P < 0.001$. n/n indicates total number of patients of vertical row/total patients of horizontal row.

* $P = 0.05$

† $P = 0.01$

‡ $P = 0.001$

Discussion

In this retrospective study, we demonstrate that SRS is additional in the diagnostic work-up and more sensitive than conventional imaging in sarcoidosis patients. The observations underscore that sarcoidosis is a systemic disease that can be visualized by this technique.

The characteristic panda sign with lacrimal and parotid glands uptake and abundant hilar, mediastinal or lung parenchyma involvement observed in the present study (Table 3) is in line with the literature (15). The remarkable high percentage of 33% positive salivary glands indicates that sarcoidosis could be present without clinical visible signs, e.g. enlarged salivary glands (13).

Hilar and/or mediastinal lymph node involvement observed on chest X-ray or CT-scan could be confirmed by SRS in every case in this study. SRS revealed significantly more thoracic lesions than conventional radiologic scans, substantiating previous but smaller studies (8). Our observations hence indicate that sarcoidosis patients may remain undetermined with conventional imaging and underscore the assumption that negative conventional imaging for sarcoidosis does not exclude pulmonary involvement.

SRS may be a tool to monitor efficacy of treatment in sarcoidosis, however patients on steroids must be excluded since treatment with corticosteroids down regulates the sst2 receptor and half of our patients on steroids still demonstrate SRS uptake (16). Five of the 63 patients with lung parenchyma uptake on SRS developed fibrosis after SRS. All 5 had no signs of pulmonary sarcoidosis on the conventional techniques, but all had signs of pulmonary involvement on SRS. To establish if certain patterns on SRS are predictive for developing fibrosis in sarcoidosis, more numbers need to be included. However, although the number of patients with diffuse lung involvement is limited in our study, our data could suggest that without lung parenchyma uptake on SRS, no pulmonary fibrosis will occur.

SRS is superior to conventional imaging and can therefore be of use as an additive tool in the detection and also in monitoring of patients suspected and treated for sarcoidosis. It might be of interest to extrapolate scintigraphic observations with bio-immunological markers, such as soluble IL-2 receptors or CD subset-analyses, in order to define the extent of disease and predict therapeutic success (17, 18).

References

1. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361:1111-1118.
2. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
3. Lichtenauer-Kaligis EG, Dalm VA, Oomen SP, et al. Differential expression of somatostatin receptor subtypes in human peripheral blood mononuclear cell subsets. Eur J Endocrinol. 2004;150:565-577.
4. Ten Bokum AM, Hofland LJ, de Jong G et al. Immunohistochemical localization of somatostatin receptor sst2A in sarcoid granulomas. Eur J Clin Invest. 1999;29:630-636.
5. Dalm VA, van Hagen PM, van Koetsveld PM, et al. Expression of somatostatin, cortistatin, and somatostatin receptors in human monocytes, macrophages, and dendritic cells. Am J Physiol Endocrinol Metab. 2003;285:E344-353.
6. Khan MU, Khan S, El-Refaie S, et al. Clinical indications for Gallium-68 positron emission tomography imaging. Eur J Surg Oncol. 2009;35:561-567.
7. Kwekkeboom DJ, Krenning EP. Somatostatin receptor imaging. Semin Nucl Med. 2002;32:84-91.
8. Kwekkeboom DJ, Krenning EP, Kno GS, et al. Somatostatin receptor imaging in patients with sarcoidosis. Eur J Nucl Med. 1998;25:1284-1292.
9. Piotrowski WJ, Bieńkiewicz M, Frieske I, et al. Somatostatin receptor scintigraphy in sarcoidosis: relation to selected clinical and laboratory markers. Pol Arch Med Wewn. 2012;122:98-106.
10. Statement on sarcoidosis. Joint Statement of the Amerian Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med. 1999;160:736-755.
11. Baughman RP, Teirstein AS, Judson MA, et al. Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001;164:1885-1889.
12. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. Br Med J 1961;2:1165-1172.
13. Baughman RP, Teirstein AS, Judson MA, et al. Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001;164:1885-1889.
14. Judson MA. A proposed solution to the clinical assessment of sarcoidosis: the sarcoidosis three-dimensional assessment instrument (STAI). Med Hypotheses. 2007;68:1080-1087.
15. Kurdziel KA. The panda sign. Radiology. 2002;215:884-885.
16. de Bruin C, Feelders RA, Waaijers AM, et al. Differential regulation of human dopamine D2 and somatostatin receptor subtype expression by glucocorticosteroids in vitro. J Mol Endocrinol. 2009;42:47-56.
17. Kamphuis LS, van Zelm MC, Lam KH, et al. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? Am J Respir Crit Care Med. 2013;187:406-416.
18. Kamphuis LS, Lam-Tse WK, Dik WA, et al. Efficacy of adalimumab in chronically active and symptomatic patients with sarcoidosis. Am J Respir Crit Care Med. 2011;184:1214-1216.

Chapter 4

Therapy

4.1

Calcium and vitamin D in sarcoidosis: is supplementation safe?

Kamphuis LS, Bonte-Mineur F, van Laar JA, van Hagen PM, van Daele PL

J Bone Miner Res. 2014;29:2498-2503

Abstract

Granulomas in sarcoidosis express high levels of 1α-hydroxylase, an enzyme that catalyzes the hydroxylation of 25-OH vitamin D to its active form, 1,25(OH)2 vitamin D. Overproduction of 1α-hydroxylase is held responsible for the development of hypercalcemia in sarcoidosis patients. Corticosteroids are used as first-line treatment in organ-threatening sarcoidosis. In this light, osteoporosis prevention with calcium and vitamin D (CAD) supplementation is often warranted. However, sarcoidosis patients are at risk for hypercalcemia, and CAD supplementation affect the calcium metabolism. We studied calcium and vitamin D disorders in a large cohort of sarcoidosis patients and investigated if CAD supplementation is safe.

Methods

Retrospectively, data of 301 sarcoidosis patients from July 1986 to June 2009 were analyzed for serum calcium, 25-hydroxy vitamin D (25-(OH)D), 1,25-dihydroxy vitamin D (1,25(OH)2D), and use of CAD supplementation. Disease activity of sarcoidosis was compared with serum levels of vitamin D.

Results

Hypercalcemia occurred in 8%. A significant negative correlation was found between 25-(OH)D and disease activity of sarcoidosis measured by somatostatin receptor scintigraphy. In our study, 5 of the 104 CAD supplemented patients developed hypercalcemia, but CAD supplementation was not the cause of hypercalcemia. Patients without CAD supplementation were at higher risk for developing hypercalcemia. During CAD supplementation, no hypercalcemia developed as a result of supplementation. Hypovitaminosis D seems to be related with more disease activity of sarcoidosis and, therefore, could be a potential risk factor for disease activity of sarcoidosis. Thus, vitamin D-deficient sarcoidosis patients should be supplemented.

Introduction

Sarcoidosis is a multisystem disease of unknown cause characterized by the presence of activated T cells, mononuclear phagocytes and non-caseating granulomas (1). Most patients with sarcoidosis do not need treatment. However in cases of organ-threatening sarcoidosis, corticosteroids are used as first-line treatment (1). Osteoporosis prevention with bisphosphonates and/or calcium and vitamin D (CAD) supplementation is advised in patients starting a course of daily 7.5 mg prednisone or higher for 3 months or longer (2).

In 1939 Harrell et al. first described hypercalcemia in sarcoidosis (3). The prevalence of hypercalcemia in patients with sarcoidosis is approximately 5 to 11% (1, 4, 5). Activated macrophages in sarcoid granulomas are capable to upregulate extrarenal 1α-hydroxylase, the enzyme which converts 25-hydroxy vitamin D (25-(OH)D) to its active form, 1,25-dihydroxy vitamin D (1,25(OH)2D). This can result in hypercalcemia (6). Other mechanisms for hypercalcemia in sarcoidosis are expression of parathyroid hormone-related protein (PTH-rP) in sarcoid macrophages what may exert an autocrine action of 1 α-hydroxylase activity and increased levels of serum interferon (IFN)-γ (7-10). IFN-γ stimulates the production of 1,25(OH)2D by alveolar macrophages (11).

In this retrospective study we examined in a large group of sarcoidosis patients the levels of calcium and vitamin D, its association with disease activity and we investigated whether CAD supplementation increased the risk of hypercalcemia.

Material and methods

Patients

This retrospective study included all sarcoidosis patients who visited the Clinical Immunology outpatient clinic in the Erasmus University Medical Center between July 1986 and June 2009. A total of 301 patients were included. Sarcoidosis was diagnosed according to the guidelines of the American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders statement on sarcoidosis (12). Data was collected using the computerised hospital information system and medical records. Patients were studied until last visit, loss to follow-up or death.

Laboratory analysis

Serum levels of calcium, 25-(OH)D and 1,25(OH)2D were measured in respectively 293, 183 and 172 samples obtained from sarcoidosis patients at moment of diagnosis. Serum calcium levels were corrected for serum albumin. Serum levels of 25-(OH)D and 1,25(OH)2D were measured using radioimmunoassays (IDS Ltd, Boldon, UK).

Imaging techniques

Disease activity of sarcoidosis was measured by chest X-ray and somatostatin receptor scintigraphy (SRS), in respectively 111 and 70 patients. Chest X-rays were classified according the Scadding system in five stages (13, 14). Stage 0, normal appearance; stage 1, bilateral hilar lymphadenopathy alone; stage 2, bilateral hilar lymphadenopathy and parenchymal shadowing; stage 3, parenchymal shadowing alone; stage 4, fibrosis. SRS uptake was graded on a four-point scale: 0, no uptake; 1, intensity less than that of the liver; 2, intensity identical to that of the liver; 3, intensity greater than that of the liver (15).

CAD supplementation

Patients treated with CAD supplementation were retrospectively selected from the included patients. The doses of CAD supplementation varied, but most patients used 500 mg calcium and 400 IU vitamin D daily. Of those patients disease activity of sarcoidosis, serum levels of calcium, 25-(OH)D and 1,25(OH)2D and possible malignancies were retrieved from the medical record.

Statistics

Statistical analyses were performed using the Pearson correlation method, one-way ANOVA test and Fisher's exact test using SPSS version 20.0. A P-value < 0.05 was considered statistically significant.

Results

In this retrospective study, 301 sarcoidosis patients (174 women and 127 men) were included.

Of the 301 patients, 293 patients had serum calcium measured at the time of diagnosis. Hypercalcemia was found in 23 patients (8%) and 4 of those 23 patients had high levels of 1,25(OH)2D.

Mean levels of 25-(OH)D did not significantly differ between the hypercalcemic and non-hypercalcemic patients, respectively 43 nmol/l and 47 nmol/l, p = 0.58). 27 patients had hypocalcemia, 17 of those 27 patients had low levels of 25(OH)D and none had low levels of 1,25(OH)2D. Serum 25-(OH)D levels were low in 115 of the 183 patients (63%). No patient had 25-(OH)D levels above the upper level of normal. In 172 patients serum levels of 1,25(OH)2D were measured at diagnosis. In 14 patients (8%) serum levels of 1,25(OH)2D were elevated. Five patients (3%) had low active vitamin D levels. Hypercalciuria, defined as a urinary calcium excretion > 5.0 mmol/l, was found in 24 patients (27%) of the 89 patients. Data are listed in Table 1A and 1B.

Table 1A. Patients characteristics of 301 sarcoidosis patients and results of measurements.

	Number of patients	Prevalence (%)
Sex		
Female	174	58
Male	127	42
Total measured serum calcium	293	
Normocalcemia	243	83
Hypercalcemia	23	8
Hypocalcemia	27	9
Total measured urine calcium	89	
Normocalciuria	65	73
Hypercalciuria	24	27
Total measured 25-(OH)D	183	
Normal 25-(OH)D	68	37
Low 25-(OH)D	115	63
Total measured 1,25(OH)2D	172	
Normal 1,25(OH)2D	153	89
High 1,25(OH)2D	14	8
Low 1,25(OH)2D	5	3

Measurements of various calcium related parameters in 301 patients diagnosed with sarcoidosis. Samples were taken at moment of diagnosis.

Serum levels of calcium were not significantly correlated with either 25-(OH)D, nor 1,25(OH)2D levels (r = -0.05, p = 0.52 and r = 0.14, p = 0.07 respectively). A significant relation was found between serum levels of 25(OH)D and 1,25(OH)2D, respectively r = 0.36, p < 0.001 (Figure 1).

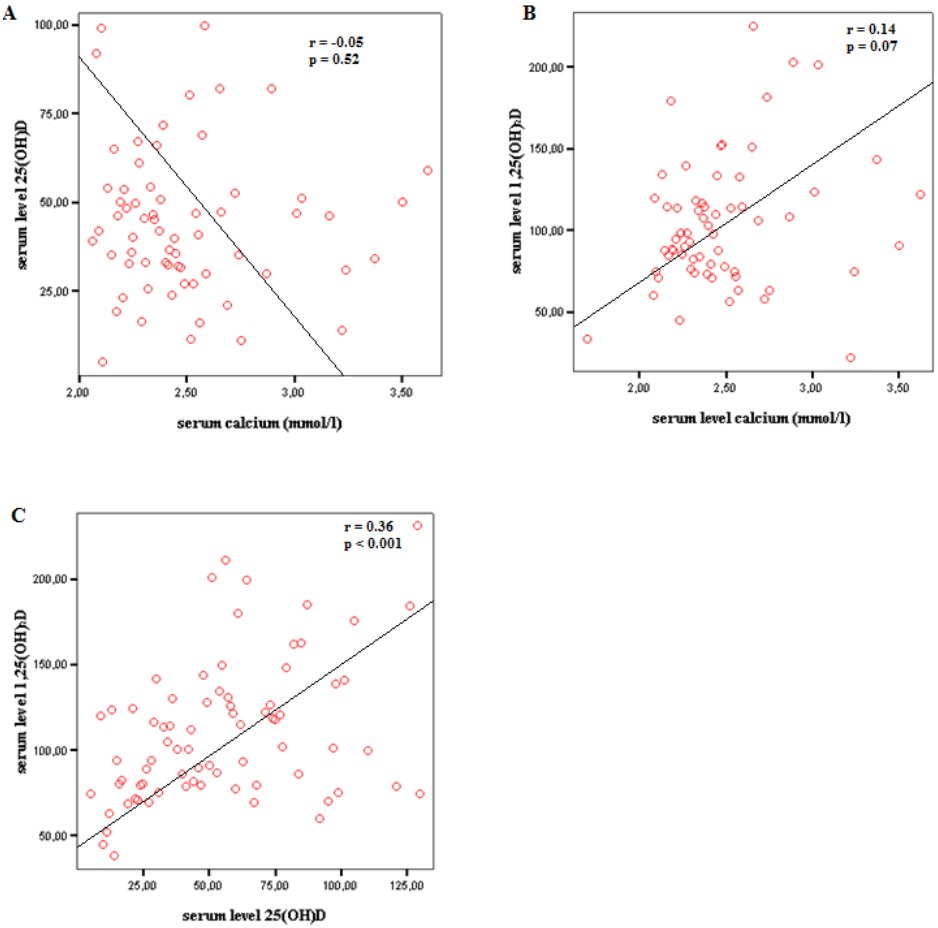
Table 1B. Patients characteristics of 301 sarcoidosis patients and results of measurements.

	Number of patients	Mean value (95% confidence interval)
Total group	301	
Calcium	293	2.35 mmol/l (2.33 – 2.37)
25-(OH)D	183	46 nmol/l (43 – 50)
1,25(OH)2D	172	111 pmol/l (104 – 117)
CAD group	104	
Calcium	67	2.39 mmol/l (2.25 – 2.52)
25-(OH)D	59	42 nmol/l (29 – 56)
1,25(OH)2D	53	114 pmol/l (99 – 129)
Not CAD group	197	
Calcium	226	2.34 mmol/l (2.29 – 2.38)
25-(OH)D	124	46 nmol/l (39 – 49)
1,25(OH)2D	119	112 pmol/l (102 - 121)
CC group	182	
Calcium	184	2.38 mmol/l (2.30 – 2.46)
25-(OH)D	104	47 nmol/l (37 – 57)
1,25(OH)2D	98	109 pmol/l (93 – 125)
Not CC group	119	
Calcium	109	2.34 mmol/l (2.29 – 2.39)
25-(OH)D	79	46 nmol/l (42 – 53)
1,25(OH)2D	74	111 pmol/l (94 – 120)

Normal values: serum calcium, 2.20-2.65 mmol/l; 25-(OH)D: 50-136 nmol/l; 1,25(OH)2D: 38-183 pmol/l.

No correlation was found between serum levels of calcium and sarcoidosis stadium on chest X-ray. Notably, a significant negative relation was found between serum level of 25-(OH)D and uptake on SRS, $p < 0.001$ (one-way ANOVA, Figure 2).

Figure 1. Correlations of the calcium and vitamin D metabolism in sarcoidosis patients.

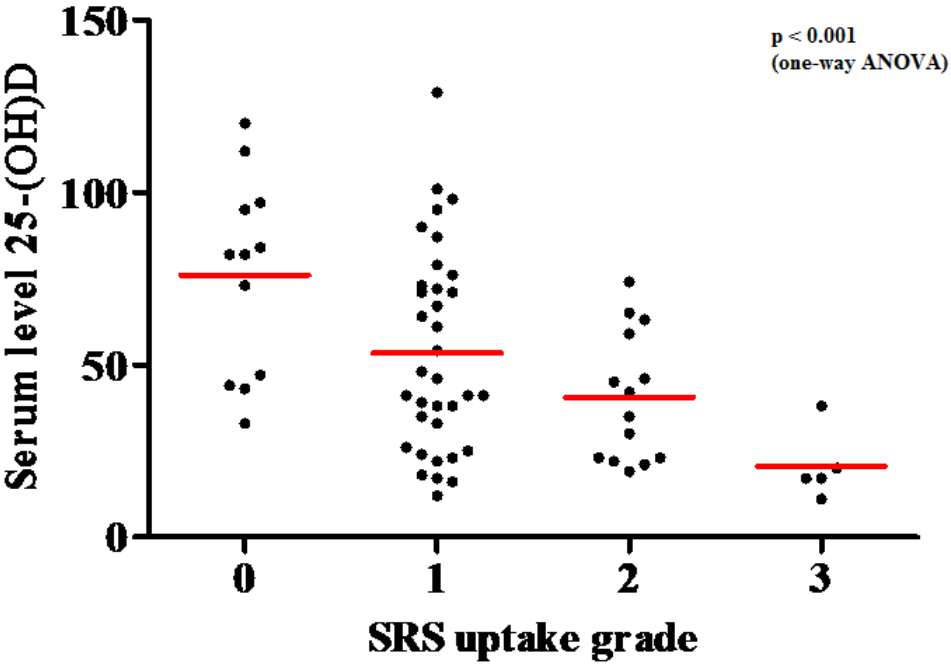


A. In 183 patients no correlation between serum level calcium and 25-(OH)D was found (r = -0.05, p = 0.52).

B. In 172 patients no statistically significant correlation was found for serum level calcium and 1,25(OH)2D (r = 0.14, p = 0.07).

C. A significant relation was found between serum levels of 25(OH)D and 1,25(OH)2D (r = 0.36, p < 0.001).

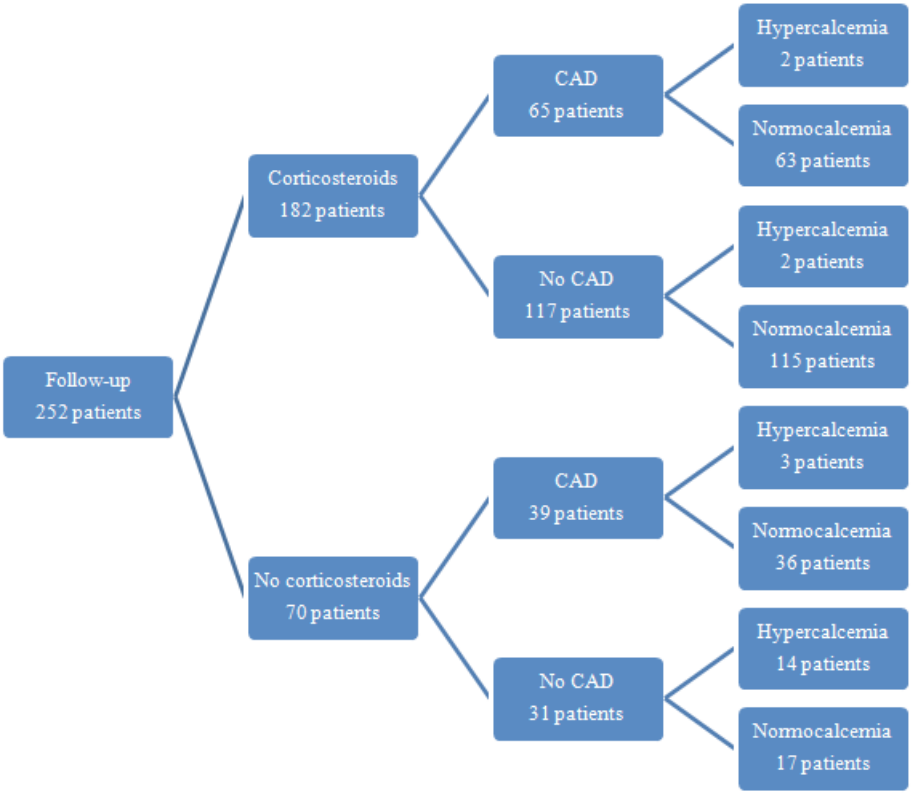
Figure 2. Correlation between serum 25-(OH)D and SRS uptake grade.



A significant correlation (one-way ANOVA) was found between uptake grade of SRS and serum level 25-(OH)D (p = 0.016).
Uptake of radioactivity in disease-related areas was graded on a four-point scale: 0, no uptake; 1, intensity less than that of the liver; 2, intensity identical to that of the liver; 3, intensity greater than that of the liver
Normal value: 25-(OH)D, 50-136nmol/L.

During follow-up 182 patients were treated with corticosteroids. Of these 182, a total of 65 patients received CAD supplementation (average dose of CAD supplementation 1.25g/400IE). Additionally, 39 patients received CAD supplementation without corticosteroid treatment (Figure 3). Of the 104 patients treated with CAD, 5 patients developed hypercalcemia. Three of these 5 patients had evidence of exacerbation of sarcoid-activity at time of diagnosis of hypercalcemia. One patient had hypercalcemia due to primary hyperparathyroidism and in one patient CAD supplementation was started at moment of hypercalcemia resulting in a more severe hypercalcemia. None of those 5 patients had elevated 25-(OH)D or 1,25(OH)2D levels (Table 2).

Figure 3. Flow chart of safety of CAD supplementation in sarcoidosis patients.



Using the Fisher's exact test for developing hypercalcemia during CAD supplementation a p-value for sarcoidosis patients treated with corticosteroids of $p = 0.61$ and a p-value of $p < 0.001$ for patients without corticosteroids treatment was found.

An additional 99 patients with CAD supplementation never developed hypercalcemia during follow-up. Mean levels of 25-(OH)D and 1,25(OH)2D prior to CAD supplementation of the 99 patients were respectively 42 nmol/l and 114 pmol/l. 14 patients developed hypercalcemia without receiving corticosteroids and CAD supplementation.

Table 2. Patients with hypercalcemia during calcium and vitamin D supplementation.

	Calcium	PTH	25-(OH)D	1,25(OH)2D	Treatment*	Reason of hypercalcemia
1	**2.86**	0.3	**31**	75	CAD	Exacerbation of sarcoidosis
2	**3.37**	**9.7**	**34**	143	CAD	Primary hyperparathyroidism
3	**2.72**	0.7	**34**	58	CAD, CC	Hypercalcemia at start of CAD
4	**3.62**	0.3	59	122	CAD, HCQ	Exacerbation of sarcoidosis
5	**3.12**	n.d.	**44**	**22**	CAD, CC	Exacerbation of sarcoidosis

Average dose of calcium and vitamin supplementation of the above mentioned patients is 1.25g/400IE. Definition of abbreviations: CAD = calcium and vitamin D supplementation; CC = corticosteroids; HCQ = hydroxychloroquine; PTH = parathyroid hormone; n.d. = not done.
Normal values: calcium = 2.20-2.65 mmol/l; PTH = 1.4-7.3 pmol/l; 25(OH)D = 50-136 nmol/l; 1.25-(OHD)2D = and 38-183 pmol/l.
Bold = abnormal values; * = treatment at moment of diagnosis hypercalcemia.
Serum calcium level is corrected for albumin.

An additional 99 patients with CAD supplementation never developed hypercalcemia during follow-up. Mean levels of 25-(OH)D and 1,25(OH)2D prior to CAD supplementation of the 99 patients were respectively 42 nmol/l and 114 pmol/l. 14 patients developed hypercalcemia without receiving corticosteroids and CAD supplementation. Mean levels of 25(OH)D and 1,25(OH)2D of the 14 patients who developed hypercalcemia without receiving corticosteroids and CAD supplementation were respectively 53 nmol/l and 178 pmol/l. The non-hypercalcemic patients without receiving corticosteroids and CAD supplementation had mean levels of 48 nmol/l and 108 pmol/l. In the whole non-hypercalcemic group mean levels of 25(OH)D and 1,25(OH)2D were 82 nmol/l and 151 pmol/l.

The 104 patients with CAD supplementation had mean levels of 25-(OH)D of 46 nmol/l prior to supplementation. Low levels of 25-(OH)D were found in 66 patients. In total the serum level of 25-(OH)D increased by 28nmol/l in the total group of 104 CAD patients. In Figure 3 a flow chart of CAD supplementation safety in sarcoidosis patients is shown. There was no significant difference in the risk of developing hypercalcemia between the patients using CAD as compared to those not using CAD.

Discussion

In this retrospective study, we examined the prevalence of calcium and vitamin D disorders in sarcoidosis patients and its association with disease activity. Furthermore we investigated if CAD supplementation is safe in patients diagnosed with sarcoidosis.

Hypercalcemia frequently occurs in sarcoidosis (4, 5). In earlier reports of Conron et al. and Adler et al., it is estimated that hypercalcemia occurs in 5 to 10 percent (4, 5, 16). In our cohort a comparable number of 8% was found.

Remarkable was the high number of patients with decreased serum levels of 25-(OH)D (63%). Although there is no consensus on optimal serum levels of 25-(OH)D. Vitamin D deficiency is defined by most experts as a 25-(OH)D serum level of less than 50 nmol/l, as was in our study (17). Sarcoidosis is most frequently diagnosed in the winter months, when vitamin D levels are at their lowest (18). The value of serum level 25-(OH)D in our study was measured at moment of diagnosis, thus mostly in the winter. However, a recent study of Powe et al. investigated the role of free 25-(OH)D (19). In this study it was concluded that levels of free 25-(OH)D were more correlated with the actual vitamin D deficient states than 25-(OH)D. Thus the total number of patients with decreased serum levels of 25-(OH)D found in our study could be lower.

Recent studies show that decreased levels of serum 25-(OH)D are a risk factor for higher disease activity in several inflammatory diseases such as Crohn's disease and multiple scleroris (17, 20, 21). In our study disease activity of sarcoidosis measured by SRS correlated negatively with serum levels of 25-(OH)D. Vitamin D is an important immunomodulator that may have a positive effect in patients with sarcoidosis (22). Thus low serum level of 25-(OH)D could be a potential risk factor for disease activity in sarcoidosis and supplementation of vitamin D in cases of deficiency could be beneficial.

In our study 104 patients were on CAD supplementation. Only 5 of the 104 CAD suppleted patients developed hypercalcemia and none of these 5 had elevated 1,25(OH)2D levels. So CAD supplementation appears to be safe. This is in accordance with much smaller studies by the group of Adler et al. who analyzed 26 patients with sarcoidosis on CAD supplementation and a recent study of Bolland et al. in which 27 sarcoidosis patients were analyzed (5, 16). Different from our study, all patients in Adler's study were Afro-Americans. Afro-Americans have a higher prevalence of low serum 25-(OH)D levels than Caucasians and the possibility to develop hypercalcemia for a Afro-American is lower (23).

In our study most patients were Caucasian, a group who is more at risk for developing hypercalcemia (23). Of the 5 patients who developed hypercalcemia during CAD supplementation, 4 patients had this event without having primary hyperparathyroidism. 3 of those 4 patients were vitamin D deficient and 2 received corticosteroids at the same time. The use of corticosteroids may prevent increases in 1,25-(OH)2D in response to CAD supplementation. Thus in patients who are receiving CAD supplementation and corticosteroids at the same time, levels of serum calcium could higher than measured.

In our study sarcoidosis patients without CAD supplementati on were at higher risk for developing hypercalcemia, without having a significant difference of serum level of 25-(OH)D between the CAD and nonCAD treated Group, (Figure 3). As mentioned before, vitamin D deficiency could possibly increase the disease activity of sarcoidosis. Thus the results of our study suggest that vitamin D deficient sarcoidosis patients should be suppleted with CAD to potentially lower disease activity.

Hypovitaminosis D and osteoporosis prophylaxis are the main reasons to supply sarcoidosis patients with CAD. Vitamin D intoxication is extremely rare but can be caused by high doses of vitamin D and induces hypercalcemia (17). The recommended daily intake of vitamin D is 200IU daily for adults up to 50 years of age, 400 IU for adults 51 to 70 years of age and 600 IU for adults 71 years of age or older (17). Doses of more than 50,000 IU per day are associated with hypercalcemia (17). Vitamin D doses of 10,000 IU per day for a period of 5 months resulted not in toxicity (24). Patients in our study used mostly 400 IU daily of vitamin D. Thus vitamin D intoxication is not expected if normal doses of CAD supplementation are used.

In conclusion our study demonstrates that serum levels of 25-(OH)D are negatively related with disease activity of sarcoidosis and that CAD supplementation is safe for sarcoidosis patients. These results indicate that lower serum levels of 25-(OH) seems to be associated with higher disease activity of sarcoidosis. Therefore in case of vitamin D deficiency, CAD supplementation should be started. Our study provides a rationale for a systematic study on vitamin D therapy in sarcoidosis patients with active disease and vitamin D deficiency.

References

1 Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
2 Majumdar SR, Lix LM, Yogendran M, Morin SN, Metge CJ, Leslie WD. Population-based trends in osteoporosis management after new initiations of long-term systemic glucocorticoids (1998-2008). J Clin Endocrinol Metabol. 2012;97:1236-1242.
3 Harrell GT, Fisher S. Blood Chemical Changes in Boeck's Sarcoid with Particular Reference to Protein, Calcium and Phosphatase Values. J Clin Invest. 1939;18:687-693.
4 Conron M, Young C, Beynon HL. Calcium metabolism in sarcoidosis and its clinical implications. Rheumatology (Oxford). 2000;39:707-713.
5 Adler RA, Funkhouser HL, Petkov VI, Berger MM. Glucocorticoid-induced osteoporosis in patients with sarcoidosis. Am J Med Sci. 2003;325:1-6.
6 Adams JS, Singer FR, Gacad MA, Sharma OP, Hayes MJ, Vouros P, et al. Isolation and structural identification of 1,25-dihydroxyvitamin D3 produced by cultured alveolar macrophages in sarcoidosis. J Clin Endocrinol Metab. 1985;60:960-966.
7 Reichel H, Koeffler HP, Barbers R, Norman AW. Regulation of 1,25-dihydroxyvitamin D3 production by cultured alveolar macrophages from normal human donors and from patients with pulmonary sarcoidosis. J Clin Endocrinol Metab. 1987;65:1201-1209.
8 Falk S, Kratzsch J, Paschke R, Koch CA. Hypercalcemia as a result of sarcoidosis with normal serum concentrations of vitamin D. Med Sci Monit. 2007;13:CS133-136.
9 Inui N, Murayama A, Sasaki S, Suda T, Chida K, Kato S, et al. Correlation between 25-hydroxyvitamin D3 1 alpha-hydroxylase gene expression in alveolar macrophages and the activity of sarcoidosis. Am J Med. 2001;110:687-693.
10 Zeimer HJ, Greenaway TM, Slavin J, Hards DK, Zhou H, Doery JC, et al. Parathyroid-hormone-related protein in sarcoidosis. Am J Pathol. 1998;152:17-21.
11 Koeffler HP, Reichel H, Bishop JE, Norman AW. Gamma-Interferon stimulates production of 1,25-dihydroxyvitamin D3 by normal human macrophages. Biochem Biophys Res Commun. 1985;127:596-603.
12 Statement on sarcoidosis. Joint Statement of the Amerian Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med. 1999;160:736-755.
13 Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med. 2001;164:1885-1889.
14 Scadding JG. Prognosis of intrathoracic sarcoidosis in England. Br Med J. 1961;2:1165-1172.
15 Kwekkeboom DJ, Krenning EP, Kno GS, Breeman WA, van Hagen PM. Somatostatin receptor imaging in patients with sarcoidosis. Eur J Nucl Med. 1998;25:1284-1292.
16 Bolland MJ, Wilsher ML, Grey A, Horne AM, Fenwick S, Gamble GD, Reid IR. Randomised controlled trial of vitamin D supplementation in sarcoidosis. BMJ Open. 2013;3:e003562.
17 Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-291.
18 Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med. 1997;336:1224-1235.
19 Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, Wenger J, Karumanchi SA, Thadhani R, Bhan I. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. J Bone Miner Res. 2011;26:1609-1616.

20 Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296:2832-2838.
21 Burke RR, Rybicki BA, Rao DS. Calcium and vitamin D in sarcoidosis: how to assess and manage. Semin Respir Crit Care Med. 2010;31:474-484.
22 Richmond BW, Drake WP. Vitamin D, innate immunity, and sarcoidosis granulomatous inflammation: insights from mycobacterial research. Curr Opin Pulm Med. 2010;16:461-464.
23 Zadshir A, Tareen N, Pan D, Norris K, Martins D. The prevalence of hypovitaminosis D among US adults: data from the NHANESIII. Ethn Dis. 2005;15:97S-101S.
24 Vieth R. Why the optimal requirement for Vitamin D3 is probably much higher than what is officially recommended for adults. J Steroid Biochem Mol Biol. 2004;89-90:575-579.

4.2

Parts of this introduction are based on:
Efficacy of adalimumab in chronically active and symptomatic patients with sarcoidosis

Kamphuis LS, Lam-Tse WK, Dik WA, van Daele PL, van Biezen P, Kwekkeboom DJ, Kuijpers RW, Hooijkaas H, van Laar JA, Bastiaans J, Baarsma GS, van Hagen PM

Am J Respir Crit Care Med. 2011;184:1214-1216

Abstract

Tumor necrosis factor-α (TNF-α) is associated with inflammatory symptoms of sarcoidosis patients.

Objectives

To investigate clinical and biochemical effects of TNF-α blocking by adalimumab in chronically active systemic sarcoidosis patients.

Material and methods

In this observational study, five patients treated with 40 mg adalimumab every other week were studied throughout 12 weeks. All patients had chronic and active biopsy-proven systemic sarcoidosis averaging ten years.

Results

Therapeutic efficacy was monitored by computered tomography (CT)-scan, somatostatin receptor-scintigraphy (SRS), serum cytokine levels and questionnaires. Within 12 weeks adalimumab treatment yielded objective improvement in four out of five patients. Lymph nodes volumes on CT-scan decreased more than 15%. The kinetics of labeled somatostatin in lymph nodes on SRS tended to an attenuation of interferon-γ and interleukin-8. The questionnaires showed a significant improvement of fatigue.

Conclusions

Four out of five patients responded to adalimumab treatment within 12 weeks. This is the first study demonstrating both clinical and biochemical improvement after adalimumab in chronic sarcoidosis.

Introduction

Sarcoidosis is a multisystem granulomatous immune mediated inflammatory disorder of unknown etiology (1). It can affect any organ in various degrees of severity. In a minority of patients the disease remains chronically active. Systemic immunosuppressive therapy is required in case of significant disease, detoriation of pulmonary function, cerebral or eye involvement or arthritis (1). Chronic sarcoidosis can result in secondary fibrosis in organs that are involved and may lead to invalidating and progressive organ failure (2).

Granulomas, the pathological hallmark of patients with sarcoidosis, reflect the process of a complex inflammatory cascade. Activated macrophages and T helper (Th 1) lymphocytes are abundant and produce symptomatic (pro)-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-1 (IL-1) (2). Other cytokines (IL-2, IL-8, IL-12, IL-15 and IL-18) may also be involved (2, 3). However, it is generally assumed that TNF-α is a key cytokine responsible for most of the inflammatory symptoms in sarcoidosis patients (2, 3). To date, the use of cytokines and cytokine receptors in monitoring disease activity of sarcoidosis is not established, however elevated levels of soluble IL-2 receptor (sIL-2R) in serum and bronchoalveolar lavage have been shown to be associated with active disease (4).

The use of immunosuppressives in sarcoidosis is hampered by adverse effects and lack of specificity. Considering the presumed important pathophysiological role of TNF-α, it might be an important target to block, hence improving therapeutic efficacy (5). Indeed, several favorable case reports and two randomized controlled trials (RCT's) on TNF-α blocking agents have been published recently (6-17). The RCT of Baughman et al., describes the efficacy of infliximab in chronic sarcoidosis patients with pulmonary involvement, while a placebo-controlled study by Rossman et al., describes the effect of infliximab in patients with active pulmonary sarcoidosis (6, 7). Both studies demonstrate improvement of pulmonary function.

Infliximab is a chimeric humanized monoclonal TNF-α blocker which was administered in these studies every six-eight weeks intravenously under direct supervision by a health care team. Adalimumab, a fully human monoclonal antibody directed against TNF-α can be administered sub cutaneously hence providing an alternative and more easy treatment option in an outpatient setting (5). Adalimumab treatment showed to be successful in patients with various immune mediated disorders, including rheumatoid arthritis (RA), Crohn's disease (CD) and psoriasis.

We describe in this observational study the clinical and biochemical effects of adalimumab in patients with chronic systemic sarcoidosis evaluated with computerized tomography (CT)-scan, somatostatin receptor scintigraphy (SRS), pulmonary function tests (PFT), serum cytokine levels and questionnaires.

Methods

Five biopsy-proven patients with chronically active and symptomatic sarcoidosis were included in this case-series. The average duration of sarcoidosis at the initiation of this study was 10 years (range 2-31 years). Patients were recruited between April 1 and June 30, 2009 from the outpatient clinic, patient characteristics are presented in Table 1. Other inclusion criteria included evaluable lesions on CT-scan, positive SRS and informed consent. Tuberculosis was excluded by history, chest X-ray, purified protein derivative test (PPD) and an interferon-gamma release assay (Quantiferon) (18).

Patients were excluded when concomitant immunosuppressives were used except hydroxychloroquine. Other exclusion criteria were HIV, HBV, HCV or Treponema pallidum, opportunistic or any other severe infection in the preceding two months; a malignancy within five years; lymphoproliferative disease; other systemic diseases; NYHA class III or IV congestive heart failure. Pregnancy, nursing or planning pregnancy 38 weeks after enrollment wasn't allowed.

Sarcoidosis and CD both share similar symptoms and some identical pathological features, hence a CD schedule (baseline 160 mg, 80 mg at week 2 and from week 4 onwards 40 mg every other week) was used (19). Initially, patients were instructed by a specialized nurse in the self-administration of adalimumab at home. Following a 12 week follow-up period patients were allowed to continue for a prolonged (> one year) period according to the treating physician's decision. During the observation period, no additional therapy was started and dosages of currently used drugs were maintained.

Routine methods of evaluation included CT-scan, SRS, PFT (forced vital capacity, total lung capacity and diffusing capacity of carbon monoxide of the lung), laboratory analyses (blood cell counts, angiotensin converting enzyme (ACE), C-reactive protein (CRP), liver enzymes, albumin, calcium, lysozyme, 25-hydroxy vitamin D, 1.25-dihydroxy vitamin D, cytokines and physical examination.

Radiological assessment included volume measurements of lymph nodes on the CT-scan and grading of uptake by SRS. The last twelve years the latter method became a reliable method to

visualize granulomas in sarcoidosis in our center.20 The intensity of pathologic uptake in the SRS was graded as follows: grade 0 = negative; grade 1 = intensity less than that of the liver; grade 2 = intensity same with that of the liver; grade 3 = intensity higher than that of the liver (20). Pulmonary involvement was classified by the Scadding system (21, 22). The volumes of multiple lymph nodes at one side (e.g. mediastinal nodes) were added together. Blood tests were regularly conducted. An enzyme-linked immunosorbent assay (ELISA, Diaclone, Besançon, France) was used to detect serum levels of sIL-2R. Serum levels of other cytokines were measured with cytometric bead arrays (CBA Human Inflammation Kit: IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α and the Human Th1/Th2 cytokine Kit: IL-2, IL-4, IL-5, IL-10, TNF-α, IFN-γ).

The fatigue was scored by using the Fatigue Assessment Scale (FAS) before treatment and at week 12.

The data are presented as mean values ± standard deviation. Statistical analyses are performed using the paired samples t test (SPSS version 18.0). A P-value < 0.05 was considered statistically significant.

Table 1. Patient characteristics.

Pt	Age at Dx	Age at Tx	Gender	Race	Organ involvement	Previous medication
1	43	48	F	C	Hilar/mediastinal lymphadenopathy, lungs, skin	HCQ
2	38	39	F	B	Hilar/mediastinal lymphadenopathy, lungs, skin, eyes, nose	Steroid eye drops
3	29	40	F	B	Hilar/mediastinal lymphadenopathy, skin, parotid glands	Steroids, HCQ
4	27	58	M	C	Hilar/mediastinal lymphadenopathy, small vessel neuropathy	Steroids, HCQ
5	74	76	M	C	Hilar/mediastinal lymphadenopathy, lungs, eyes	HCQ, steroid eye drops

Abbrevations: Pt = patient; C = Caucasian; F = female; M = male; B = black; HCQ = hydroxychloroquine.

Results

Patients' characteristics are summarized in table 1. All patients had both pulmonary and extra-pulmonary localizations of the sarcoidosis.

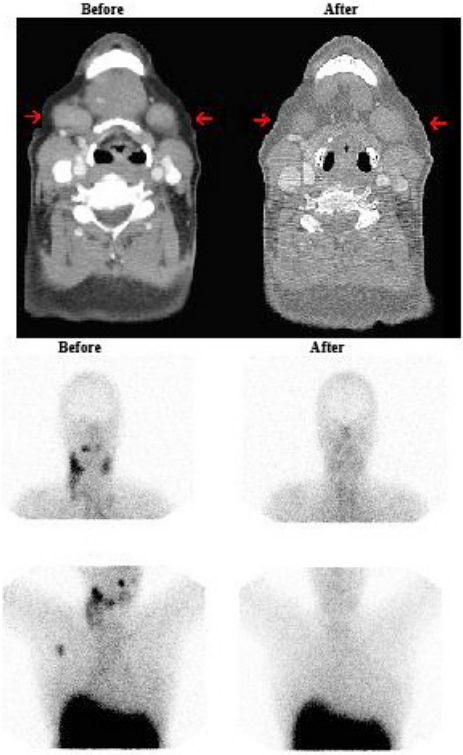
Table 2 displays the radiological findings during the observational period of 12 weeks. In four patients (patient 1-3, 5) radiologic improvement was observed. Patient 4 showed a mixed response. Enlarged thoracic and axillary lymph nodes decreased significantly (17.6% ± 14.3, P < 0.001) on the CT-scan.

Pathological uptake in sarcoidosis related lesions on the SRS decreased comparably and was felt to be even more sensitive than those of the CT-scan (Table 2 and Figure 1). Also dermatological involvement improved (Figure 2a, 2b).

PFT's of all patients were normal at initiation of therapy and remained within normal limits (data not shown).

During the observation, levels of ACE, CRP and calcium were within normal limits, whereas lysozyme levels were initially increased in patient 1, 2 and 5 (range 14-21 mg/l) before treatment and returned to normal (< 12 mg/l) during treatment. Elevated serum sIL-2R levels occurred only in two patients and were subsequently disregarded for further evaluation (data not shown). The serum IFN-γ levels were elevated before treatment in four out of five patients (range 9.8-37.5). All patients had elevated IL-8 serum levels (range 14.4-31.8) before treatment.

Figure 1.



The CT scan and SRS of a patient with chronically active sarcoidosis before ((left) and 12 weeks after (right) TNF-α blocking therapy with adalimumab. Increased uptake in nose, salivary glands, and right axillary lymph node and mild uptake in hilar lymph nodes is demonstrated with SRS before treatment (left). The SRS 12 weeks after treatment (right) displays mild uptake in the salivary glands. Note that although the size of cervical lymph nodes as displayed on CT scan remain almost equal, the uptake of these lesions on the SRS disappear.

Table 2. Changes in radiographic imaging and fatigue after 12 weeks adalimumab treatment.

Pt	Region	CT-scan before (in mm)	CT-scan after (in mm)	Change in diameter	SRS before	SRS after	FAS score before
1	Submandibular	25.1	21.2	-15.5%	1	1	35
	Hilum	23.7	15.8	-33.4%	1	0	
	Mediastinum	20.0	15.9	-20.5%	0	0	
	Axillar	16.6	10.1	-39.2%	1	0	
	Inguinal	21.4	14.8	-30.8%	1	0	
	Skin				1	0	
2	Lacrimal gland	8.7	8.4	-3.4%	3	0	37
	Nose				3	0	
	Submandibular	17.7	13.4	-24.3%	2	1	
	Parotid glands	36.9	30.0	-18.7%	2	1	
	Hilum	16.9	14.0	-17.2%	2	1	
	Mediastinum	22.4	21.0	-6.2%	1	1	
	Axillar	15.2	7.0	-53.9%	1	0	
	Inguinal	19.5	17.1	-12.3%	2	1	
	Skin				2	1	
3	Lacrimal gland	13.4	10.9	-18.7%	1	0	25
	Nose				1	1	
	Submandibular	24.1	18.3	-24.1%	1	0	
	Parotid glands	40.3	38.6	-4.2%	2	2	
	Hilum				1	1	
	Mediastinum	24.2	24.2	0%	1	1	
	Axillar				0	0	
4	Hilum	9.9	10.1	+2.0%	0	0	34
	Mediastinum	8.5	8.3	-2.4%	0	0	
	Lung parenchyma				1	1	
	Joints				1	0	
5	Submandibular	20.7	16.5	-8.5%	1	0	24
	Hilum	20.8	16.8	-19.2%	2	1	
	Mediastinum	28.2	25.8	-20.3%	1	1	
Mean		20.7	17.1	-17.7	1.24	0.52	31
SD		8.0	7.8	13.9	0.8	0.6	6.0

Abbrevations: Pt = patient; CT = computer-tomography; SRS = somatostatin receptor scintigraphy; mm = millimeter; FAS = Fatigue Assessment Scale; SD = standard deviation. Nodes identified on CT-scan are listed in diameter (mm). The largest diameter of the node was measured. The intensity of pathologic uptake in the SRS was graded as follows: grade 0 = negative; grade 1 = intensity less than the liver; grade 2 = intensity same with that of the liver; grade 3 = intensity higher than the liver. The FAS is a fatigue questionnaire consisting of 10 items; five questions reflecting physical fatigue and five questions for mental fatigue. The five point rating scale varies from 1, never, to 5, always. Scores on the FAS can range from 10 to 50.

Figure 2a. Patient 1with skin involvement of sarcoidosis before treatment with adalimumab. The patient gave permission for publishing the photos.

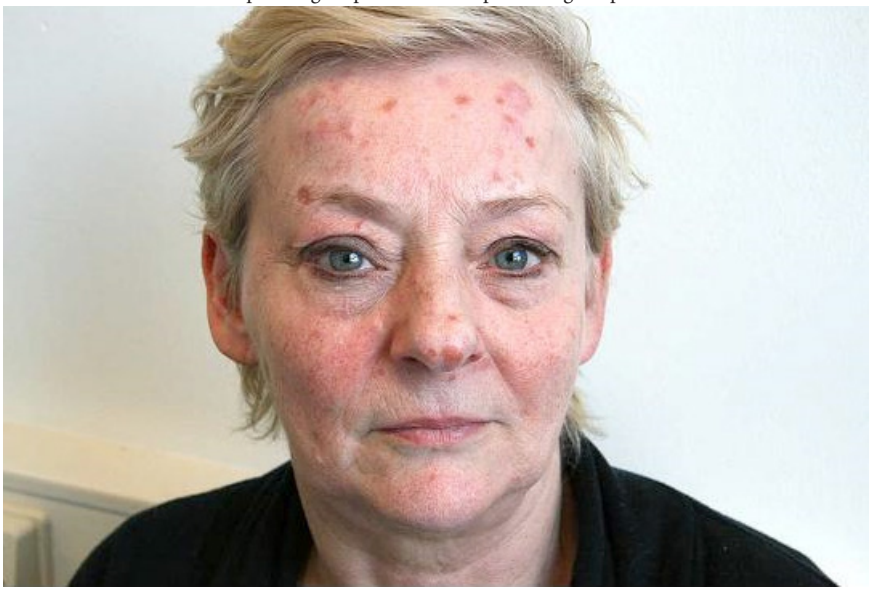
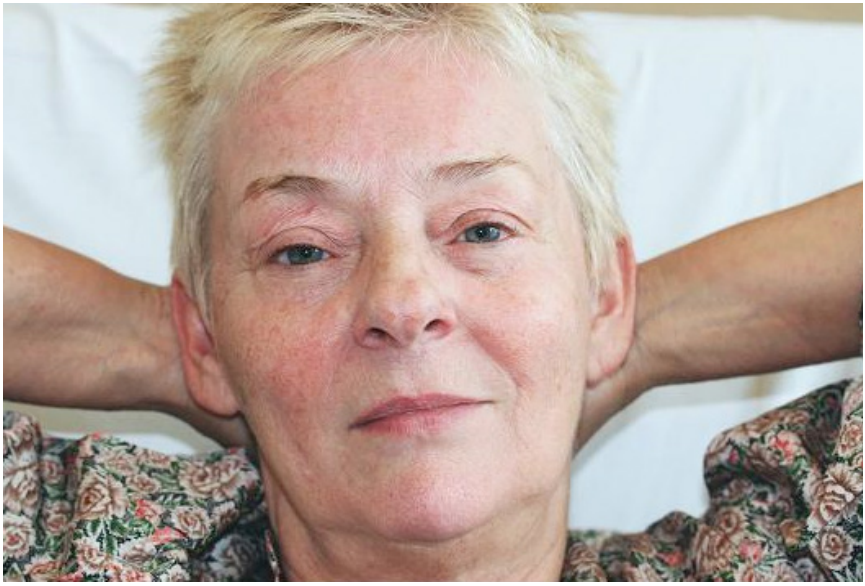


Figure 2b. Patient 1 with cutaneous sarcoidosis after 12 weeks treatment with adalimumab.



IFN-γ decreased slightly in three patients at week 12 (range 6.4-19.0). IL-8 levels decreased in three patients at week 12 (range 1.3-31.0). These observations did not reach statistical significance. The other cytokines were not significantly elevated (data not shown).

Clinical symptoms improved in four out of five patients. Improvement of fatigue (FAS score 31.0 ± 6.0 before; 22.6 ± 3.6 ($P < 0.05$) 12 weeks after treatment, table 2), was observed. Although not validated by a standardized dyspnoea score, four patients noticed a subjective improvement of dyspnoea.

Discussion

In this observational study adalimumab treatment proved beneficial within 12 weeks in four out of five patients with chronically active sarcoidosis. Clinical improvement was accompanied by the observation of decreasing lysozyme levels and -sarcoid lesions as measured by radiologic and scintigraphic techniques. Furthermore serum IFN-γ, IL-8 levels tended to correspond with clinical response. To our knowledge, this is the first study to relate therapeutic efficacy of adalimumab with several inflammatory parameters in a case series of chronic sarcoidosis patients.

The demonstrated therapeutic efficacy of adalimumab in this study is not unexpected, but up till now experience and evidence with adalimumab in sarcoidosis is limited in comparison with infliximab. TNF-α blockers, such as infliximab, etanercept and adalimumab were successfully introduced in the treatment of inflammatory disorders more than one decade ago (23). Recently, the TNF-α blocker infliximab demonstrated significant clinical benefit in large cohorts of patients with pulmonary manifestations of sarcoidosis (24, 25). In two double blinded randomized, and placebo controlled trials of patients with pulmonary sarcoidosis a favorable clinical response was demonstrated (6, 7). Etanercept has been reported effective in case reports, but the only trial so far resulted in detoriation of almost a third of the patients with acute pulmonary sarcoidosis (26-28). Adalimumab could be an important additional option in the treatment with biologicals.

There is no consensus regarding timing and duration of TNF-α blockers in rheumatological and other inflammatory diseases. For example CD patients require higher and more intensive treatment schedules of adalimumab than RA patients (29, 30).

The optimal schedule for TNF-α blockers in sarcoidosis remains unknown, according to the case studies, dosages might be similar to those used in CD (8, 10). In the current study we initiated a CD schedule and used a relatively short observation period of 12 weeks. Hereafter, all patients continued adalimumab for at least one year. In this period the patient that initially showed a mixed response (patient 4) noted a decrease in disease related symptoms, accompanied by improved radiological findings after increasing the frequency of administration after 16 weeks (data not shown). This observation stresses the importance to adjust dosing and await responses for a longer period. Furthermore, this study suggests that an effective schedule requires starting with induction of 160 mg, and 80 mg 2 weeks later, followed by 40 mg every other week. In case of no immediate –or only moderate response, dose –or frequency intensification may be considered. Three years after inclusion in this study, three patients (patient 1, 3 and 5) have successfully stopped treatment with adalimumab. After 2.5 years the response of patient 2 failed, so she switched to infliximab, another TNF-α blocker. Patient 4 is still using adalimumab once a week with a mixed response.

We have used SRS to evaluate sarcoidosis activity in vivo, although 18F fluordeoxyglucose (18F-FDG) PET is the classical scintigraphic method. In our center we have built up extensive experience with this method in various (immune mediated inflammatory) diseases (20). In the current study 18F-FDG PET was initially compared to SRS, but appeared not superior (data not shown). SRS enabled localization of granulomas by binding to the somatostatin receptors that are frequently involved in immune and inflammation responses (20, 31). The observations in this study in for example patient 1 (Figure 1), suggest that the decrease of the inflammation induced by adalimumab results in less expression of somatostatin receptors. Therefore SRS could be a useful imaginary technique to monitor organ involvement and therapeutic efficacy in sarcoidosis.

Many studies on monitoring sarcoidosis have been accomplished, but to date no easily applicable marker has emerged. A possible serological marker of sarcoidosis activity is sIL-2R (4). This cytokine is considered to reflect T-cell activation (32). Cytokines such as IL-2, but also IFN-γ and IL-8 however, are not specific for sarcoidosis and might also reflect other inflammatory processes (3). Grutters et al. described lower serum sIL-2R levels in sarcoidosis patients with persistent pulmonary lesions as compared to patients with a normalization of their chest x-ray (32). In line with this observation, sIL-2R levels and pulmonary lesions persisted in our patients. It might therefore be proposed that serum sIL-2R levels do not reflect disease

activity in patients with chronically active pulmonary sarcoidosis. Our data suggest that serum IFN-γ and IL-8 levels might be more appropriate in this setting. Decreased serum IFN-γ and IL-8 levels tended to correlate with radiologic improvement as well as improvement of clinical symptoms. This observation adds to the observations by Yokoyama et al. who correlated serum IL-8 levels with disease activity in 13 out of 16 patients with chronic sarcoidosis (33).

In conclusion, this is the first study to demonstrate that adalimumab treatment schedule in patients with systemic and chronically active sarcoidosis results in a favorable clinical outcome by means of improved radiographic scans, a trend of decreased serum cytokine levels and improvement of clinical symptoms.

References

1. Dempsey OJ, Paterson EW, Kerr KM, Denison AR. Sarcoidosis. BMJ. 2009;339:b3206.
2. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Eng J Med. 2007;357:2153-2165.
3. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361:1111-1118.
4. Bargali E, Bianchi N, Margollicci M, Olivieri C, Luddi A, Coviello G, Grosso S, Rottolli P. Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. Scand J Clin Lan Invest. 2008;68:479-483.
5. King CS, Kelly W. Treatment of sarcoidosis. Dis Mon. 2009;55:704-718.
6. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Müller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES; Sarcoidosis Investigators. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med. 2006;174:795-802.
7. Rossman MD, Newman LS, Baughman RP, Teirstein A, Weinberger SE, Miller W Jr, Sands BE. A double-blinded, randomized, placebo-controlled trial of infliximab in subjects with active pulmonary sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2006;23:201-208.
8. Philips MA, Lynch J, Azmi FH. Ulcerative cutaneous sarcoidosis responding to adalimumab. J Am Acad Dermatol. 2005;53:917.
9. Callejas-Rubio JL, Ortego-Centeno N, Lopez-Perez L, Benticuaga MN. Treatment of therapy-resistant sarcoidosis with adalimumab. Clin Rheumatol. 2006;25:596-597.
10. Heffernan MP, Smith DI. Adalimumab for treatment of cutaneous sarcoidosis. Arch Dermatol. 2006;142:17-19.
11. Thielen AM, Barde C, Saurat JH, Laffitte E. Refractory chronic cutaneous sarcoidosis responsive to dose escalation of TNF-alpha antagonists. Dermatology. 2009;219:59-62.
12. Dhingra N, Morgan J, Dick AD. Switching biologic agents for uveitis. Eye (Lond). 2009;23:1868-1870.
13. Cufi-Benet M, Sabadell C, Codina O. Adalimumab in the treatment of parotid sarcoidosis. Arch Bronconeumol. 2010;46:49-50.
14. Patel SR. Systemic sarcoidosis with bone marrow involvement responding to therapy with adalimumab: a case report. J Med Case Reports. 2009;3:8573.
15. Marnane M, Lynch T, Scott J, Stack J, Kelly PJ. Steroid-unresponsive neurosarcoidosis successfully treated with adalimumab. J Neurol. 2009;256:139-140.
16. Leo G, Iuliano A, Spina D, Bellisai F, Fioravanti A, Galeazzi M. Simultaneous pulmonary involvement, histologically proven, of sarcoidosis and rheumatoid arthritis. Clin Exp Rheumatol. 2009;27:164.
17. Field S, Regan AO, Sheahan K, Collins P. Recalcitrant cutaneous sarcoidosis responding to adalimumab but not to etanercept. Clin Exp Dermatol. 2010;35:795-796.
18. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, Siegel JN, Braun MM. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Eng J Med. 2001;345:1098-1104.
19. Simillis C, Jacovides M, Reese GE, Yamamoto T, Tekkis PP. Meta-analysis of the role of granulomas in the recurrence of Crohn disease. Dis Colon Rectum. 2010;53:177-185.
20. Kwekkeboom DJ, Krenning EP, Kno GS, Breeman WA, van Hagen PM. Somatostatin receptor imaging in patients with sarcoidosis. Eur J Nucl Med. 1998;25:1284-1292.

21. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R; Case Control Etiologic Study of Sarcoidosis (ACCESS) research group. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 2001;164:1885-1889.
22. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. Br Med J 1961;2:1165-1172.
23. Nash PT, Florin TH. Tumour necrosis factor inhibitors. Med J Aust. 2005;183:205-208.
24. Baughman RP, Costabel U, du Bois RM. Treatment of sarcoidosis. Clin Chest Med. 2008;29:533-548.
25. Baughman RP. Tumor necrosis factor inhibition in treating sarcoidosis: the American experience. Revista Portuguesa de Pneumonologia. 2007;13:47-50.
26. Khanna D, Liebling MR, Louie JS. Etanercept ameliorates sarcoidosis arthritis and skin disease. J Rheumatol. 2003;30:1864-1867.
27. Tuchinda C, Wong HK. Etanercept for chronic progressive cutaneous sarcoidosis. J Drugs Dermatol. 2006;5:538-540.
28. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. Chest. 2003;124:177-185.
29. Sandborn WJ, Hanauer SB, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh DG, Panaccione R, Wolf D, Kent JD, Bittle B, Li J, Pollack PF. Adalimumab for maintenance treatment of Crohn's disease: results of the CLASSIC II trial. Gut. 2007;56:1232-1239.
30. Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Colombel JF, Panaccione R, D'Haens G, Li J, Rosenfeld MR, Kent JD, Pollack PF. Adalimumab induction therapy for Crohn disease previously treated with infliximab: a randomized trial. Ann Intern Med. 2007;146:829-838.
31. Duet M, Lioté F. Somatostatin and somatostatin analog scintigraphy: any benefits for rheumatology patients? Joint Bone Spine. 2004;71:530-535.
32. Grutters JC, Fellrath JM, Mulder L, Janssen R, van den Bosch JM, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. Chest. 2003;124:186-195.
33. Yokoyama T, Kanda T, Kobayashi I, Suzuki T. Serum levels of interleukin-8 as a marker of disease activity in patients with chronic sarcoidosis. J Med. 1995;26:209-219.

Chapter 5

Discussion

General discussion

Sarcoidosis is a fascinating disease with many diverse clinical and immunological aspects, making it a challenge to dissect disease pathogenesis and to optimize treatment protocols.

Thus far the pathophysiology of sarcoidosis is unknown. In 21th century, an abundance of studies have demonstrated potential models for the pathogenesis. The consensus model states that activated T cells play a crucial role in the pathophysiology of sarcoidosis (1). However, all components of the immune system can be involved, in this thesis we present for the first time an immunopathophysiologal model indicating the importance of B cells in sarcoidosis as described in Chapter 2. Moreover, we evaluated new insights for diagnostic techniques (Chapter 3) and new treatment options in this thesis (Chapter 4).

Pathophysiology

In Chapter 1, the hypothesized model for immunopathogenesis of sarcoidosis is presented. Sarcoidosis is considered to be a T cell driven disease with the occurrence of noncaseating granulomas (2-4). Granuloma formation is the central immunological characteristic in the pathogenesis of sarcoidosis. Granulomas are the result of a physiological immunological response to a (low antigenic) danger signal and results in the isolation of pathogens the immune system itself cannot eliminate. Consequently, granulomas prevent the surrounding tissues from continuing inflammatory and damaging effects. The granulomas are thought to be formed by an interaction between antigen presenting cells (APC) and activated T-lymphocytes, mainly of the CD4 lineage. This inter action leads to a release of cytokines, especially interleukin (IL)-2 and interferon (IFN)-γ. These pro-inflammatory cytokines trigger macrophages to produce tumor necrosis factor-α (TNF-α) and strengthen the local inflammation response by releasing a cascade of additional cytokines (2, 5).

Apart from the APC – T lymphocyte interaction several factors suggest that the B cell response in sarcoidosis may be abnormal. These B-cell related factors include the occurrence of circulating immune complexes, hypergammaglobulinemia, autoantibody production, anergy and reduced frequencies of circulating CD27+ memory B cells (6-10). In Chapter 2, the cellular and molecular characteristics of B cells were intensively analyzed in patients with sarcoidosis. The observations suggest redistribution of memory B cells towards granulomatous tissue.

Together with the observation of increased circulating transitional B cell numbers and increased molecular signs of antibody maturation, this indicates an involvement of an overactive B cell response in the pathophysiology of sarcoidosis. Thus, patients with sarcoidosis might benefit from B cell directed therapy.

The results in Chapter 2 demonstrated that plasma cells in granulomatous tissue of sarcoidosis patients produce mostly IgA. Importantly, IgA-producing plasma cells produce TNF-α, one of the key cytokines in the pathogenesis of sarcoidosis (Chapter 4) (11). Unfortunately, we were not able to confirm this by stimulation of CD27-IgA+ memory B cells, however this could be caused by limited analytical procedures (Chapter 2). Moreover, B cells stimulate TNFR2 expression (12). There are reports of increased levels of TNFR2 in sarcoidosis patients (13). We did not investigate the production of TNFR2 after stimulating B cells, but a hypo thesis could be that CD27-IgA+ memory B cells are activating the expression of TNFR2 which stimulates the production of sarcoid granulomas.

The peripheral blood memory B cell compartment is significantly affected in patients with sarcoidosis, reduced numbers of natural effector B cells and T cell-dependent CD27+IgM+, CD27+IgG+ and CD27+IgA+ memory B cell subsets were observed (Chapter 2). Concomitant studies showed low CD27+ memory B cells in the blood of patients with severe chronic sarcoidosis (6, 14). However, by the use of more detailed analysis of we revealed that this impairment is mainly caused by decreased numbers of T cell-dependent memory B cells. In contrast, T cell-independent CD27-IgA+ memory B cells are increased in sarcoidosis patients. This could explain why the total amount of B cells is normal in patients with sarcoidosis. It remains to be determined if the increase of T cell-independent B-cell memory directly results from pathogenic B cell responses or indirectly to compensate for the loss of other memory B cells.

By use of further in depth analyses with detailed molecular analyses of IgA and IgG memory B cells we demonstrate increased somatic hypermutations levels and immunoglobulin class switching to downstream IgG subclasses in patients with sarcoidosis (Chapter 2). These molecular patterns suggest that memory B cells in sarcoidosis have been exposed to prolonged specific B cell stimulation or consecutive responses (15-17). Thus it is unlikely that sarcoidosis patients have defects in the generation of B cell memory, but rather have involvement and migration of antigen-experienced B cells towards granulomatous tissue.

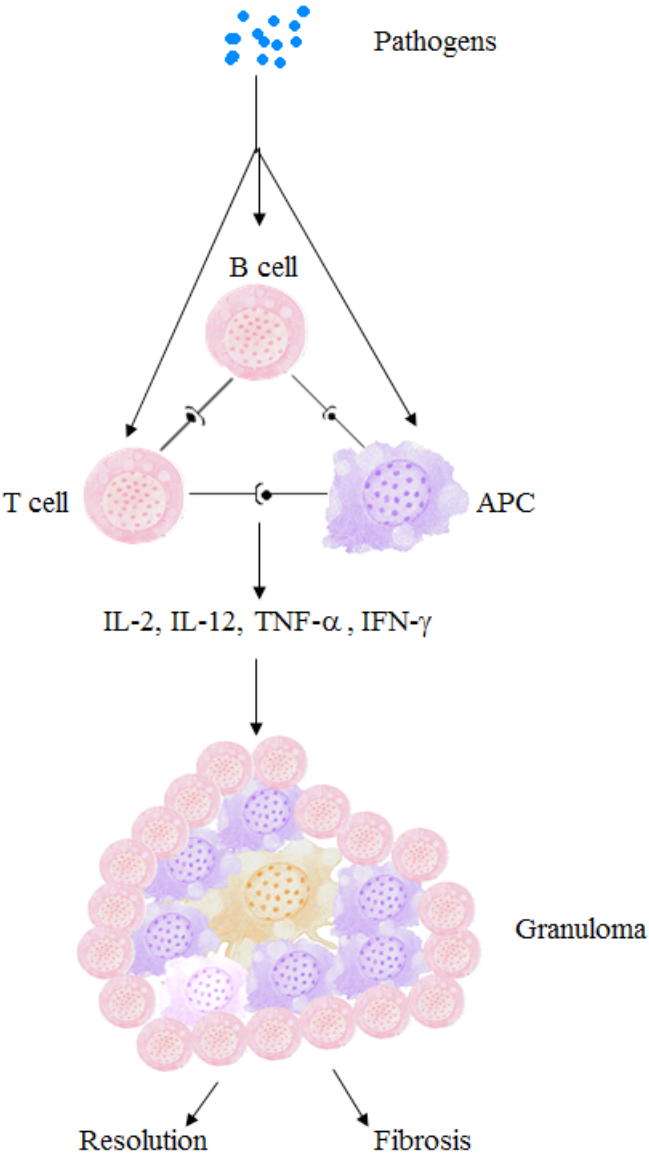
We therefore hypothesize that chronic B cell stimulation is related to formation of granulomatous tissue in a manner similar as in patients with sarcoidosis-lymphoma syndrome involving predominantly malignant B cells (16, 18-21). It is thought that these B cells undergo malignant transformation after chronic stimulation (22).

The function of B cells and their role in the formation of granulomas is further discussed in Chapter 2. Interestingly, certain patients with common variable immunodeficiency (CVID), which is known as a B cell differentiation disorder, can develop granulomas and might therefore support a theoretical pathophysiological model for sarcoidosis (61, 62). In contrast to sarcoidosis, granulomatous CVID patients have hypogammaglobulinemia (because of an impaired immunoglobulin production), but have similar reduced numbers of blood memory B cells and increased transitional B cells (15, 23, 24). We assume that it is unlikely that the decreased numbers of memory B cells are a sign of dysfunctional B cell development, because we demonstrate normal immunoglobulin responses after vaccination, circulating plasma cell numbers and normal serum immunoglobulin levels in sarcoidosis. However, B cells seem to be essential for the development of granulomas. This can be learned from patients with X-linked agammaglobulinemia (XLA), a distinct form of hypogammaglobulinemia characterized by a complete B cell deficiency. These XLA patients do not develop granulomas (25). Furthermore, in models of T cell deficient mice, it has been shown that B cells are crucial for non-infectious granuloma formation (26). Thus, B cells appear to be essential for granuloma formation in both antibody-deficient patients and in patients with sarcoidosis. Granuloma formation in CVID suggests that immature transitional B cells and immature non-switched B cells play a role in granuloma formation. It is of particular interest to identify which type of B cell subset is found in the granulomatous tissue of CVID patients as compared with sarcoid granuloma (15).

Sarcoidosis is thought to be mainly a T-helper (Th)-1 driven disease (2). The Th-2 counterpart of this spectrum of diseases is atopy. In order to study Th-2 responses clinical Th-1 and Th-2 responses were studied in sarcoidosis. We observed a Th-1 preference in sarcoidosis by demonstrating the lack of association between atopy and sarcoidosis (Chapter 2). Antagonisms between Th-1 and Th-2 axes has been linked to a lower prevalence and a less severe course of autoimmune diseases in other studies (27). In line with this, we demonstrated in Chapter 2 that atopic eczema was negatively associated with uveitis (defined as a Th-1 disease) in sarcoidosis. How an eczematous skin may contribute in the protection of physically and immunologically isolated eyes is not clear, but may be part of a generally

inhibition of Th-2 responses by Th-1. Figure 1 shows a updated version of the hypothesized pathogenesis of sarcoidosis with results of this thesis.

Figure 1. Updated version of the hypothesized pathogenesis of sarcoidosis with results of this thesis.



Diagnostics

Traditionally, sarcoidosis is diagnosed by compatible clinical and conventional radiological findings (X-ray and CT scan), the presence of non-caseating granulomas and exclusion of similarly presenting disorders (2). In Chapter 3 we show that the somatostatin receptor scintigraphy (SRS) has added value in the diagnostic workup and is more sensitive than conventional imaging in sarcoidosis patients. In this longstanding study, all patients with hilar and/or mediastinal lymph node involvement observed on chest X-ray or CT scan were confirmed by SRS. SRS revealed significantly more thoracic lesions than conventional radiologic imaging. Our observations hence indicate that the extend of sarcoid disease remains underestimated with conventional imaging and underscore the assumption that negative conventional imaging for sarcoidosis does not exclude pulmonary involvement.

Evaluation of disease activity and therapeutic monitoring is not yet standardized. Increased levels of soluble IL-2R (sIL-2R), associated with T cell activation, are associated with disease. However, this cytokine receptor is not specific for sarcoidosis and increased levels are also found in other inflammatory processes (2, 28). Paradoxically low serum sIL-2R levels are described in patients with sarcoidosis with persistent pulmonary lesions as compared with patients with chest X-ray normalization (29). In line with these observations and lack of relevant sIL-2R levels accompanying persistent pulmonary lesions in our study presented in chapter 4, we assume that sIL-2R levels do not reflect disease activity, at least not in patients with chronically active pulmonary sarcoidosis.

Because of the lack of defined disease activity parameters in sarcoidosis, peripheral blood biomarkers may be suitable alternatives. ACE is a serum factor that is related to the activity of disease in cohort studies, but lacks clinical importance in individual patients. It has been demonstrated in Chapter 2 that treatment with a TNF-α blocker normalizes the population of CD27-IgA+ memory B cells. Suggesting that CD27-IgA+ memory B cell numbers could be a potential marker of sarcoidosis activity. Another unexpected potential marker for disease activity in sarcoidosis might be 25-(OH)D levels. In Chapter 4 we described an inverse relation between disease activity of sarcoidosis measured by SRS and serum levels of 25-(OH)D. Recently, other studies have shown that decreased levels of serum 25-(OH)D are a risk factor for higher disease activity in several inflammatory diseases such as multiple sclerosis and Crohn's disease (30-32).

Therapy

The outcome of sarcoidosis is highly variable. The majority (two-third) of patients have spontaneous resolution or show a good response to first-line steroid therapy (2, 33). Osteoporosis, diabetes and infections are a few of the many effects of corticosteroids (34). Hence, osteoporosis prevention with bisphoshonates and/or calcium and vitamin D supplementation is recommended in patients using a course of daily 7.5mg prednisone or higher for a period of 3 months or longer (35). However, hypercalcemia is described in 5-11% of the sarcoidosis patients. Therefore, calcium and vitamin D supplementation is not without risk (2, 36, 37). Nevertheless, in Chapter 4 it is demonstrated that supplementation of calcium and vitamin D is relatively safe in sarcoidosis patients if there is no hypercalcemia at moment of starting the supplementation. Still, hypercalcemia can be a consequence of vitamin D therapy and has to be monitored carefully.

Sarcoidosis is most frequently diagnosed in the winter months, when serum vitamin D levels are at their lowest (38). Thus, when vitamin D levels were determined moment of diagnosis, this was mostly done in winter. This co-incidence does not explain that lowered vitamin D levels are causing sarcoidosis, but the association remains evocative (Chapter 4). If indeed decreased levels of serum 25-(OH)D are a potential risk factor for higher disease activity in sarcoidosis patients supplementation of vitamin D might not only be of prophylactic, but also of therapeutic value.

There is no defined rational therapy for sarcoidosis. Unfortunately a third of the sarcoidosis patients develop a chronic, progressive disease which can be refractory to multiple lines of treatment (2). Table 1 shows the current immunosuppressive treatment in sarcoidosis.

TNF-α inhibition in patients with sarcoidosis has been shown to reduce disease signs and symptoms (39). Multiple TNF-blockers have been produced and tested in clinical trial. In a phase II, multi-centre, randomized double-blind, placebo-controlled study of 138 patients with chronic sarcoidosis, treatment with infliximab (chimeric monoclonal antibody against TNF-α) resulted in a limited but statistically significant improvement of 2.5% of the predicted forced vital capacity (FVC) over placebo (40). Obviously, it has been shown that extrapulmonary affected organs show a better response to infliximab (41). According to this, golimumab, another monoclonal antibody against TNF-α, had no efficacy in pulmonary sarcoid lesions, but trends towards improvement in some dermatological end-points is observed (42). Etanercept, another TNFα-blocker, has been reported to be effective in several case reports.

However, the only randomized clinical trial (RCT) with this drug showed aggravation of disease in 65% of the patients after (43). Thus, this TNFα-blocker has been abandoned in the treatment of sarcoidosis (44-46). In Chapter 4 we present a case series of five patients with symptomatic, chronic, both pulmonary and extra pulmonary sarcoidosis treated with adalimumab (human monoclonal antibody against TNF-α). Radiological and clinical improvement occurred within 12 weeks. After dose intensivation in one patient all patients demonstrated improvement by improved radiological images, stressing the importance to adjust dosing in individual patients.

Table 1. Current immunosuppressive treatment in sarcoidosis.

Medication	Dose
Glucocorticosteroids	
Prednisone	start 5-40 mg/day
	maintenance <10 mg/day
Anti malaria drugs	
Hydroxychloroquine	200-400 mg/day
Cytotoxic medication	
Azathioprine	50-150 mg/day
Cyclophosphamide	50-150 mg/day oral
	500-1500 mg Q2-4W i.v.
Methothrexate	10-25 mg/weekly
Sodium mycophenolate/mycophenolate mofetil	720mg/1000mg twice daily
Cytokine modulating medication	
Infliximab	5-10 mg/kg Q4-8W
Adalimumab	40- 80 mg Q1-2W
Thalidomide	50-100 mg/day

TNF-α inhibition in patients with sarcoidosis has been shown to reduce disease signs and symptoms (39). Multiple TNF-blockers have been produced and tested in clinical trial. In a phase II, multi-centre, randomized double-blind, placebo-controlled study of 138 patients with chronic sarcoidosis, treatment with infliximab (chimeric monoclonal antibody against TNF-α) resulted in a limited but statistically significant improvement of 2.5% of the predicted forced vital capacity (FVC) over placebo (40). Obviously, it has been shown that extrapulmonary affected organs show a better response to infliximab (41).

According to this, golimumab, another monoclonal antibody against TNF-α, had no efficacy in pulmonary sarcoid lesions, but trends towards improvement in some dermatological end-points is observed (42). Etanercept, another TNFα-blocker, has been reported to be effective in several case reports. However, the only randomized clinical trial (RCT) with this drug showed aggravation of disease in 65% of the patients after (43). Thus, this TNFα-blocker has been abandoned in the treatment of sarcoidosis (44-46). In Chapter 4 we present a case series of five patients with symptomatic, chronic, both pulmonary and extra pulmonary sarcoidosis treated with adalimumab (human monoclonal antibody against TNF-α). Radiological and clinical improvement occurred within 12 weeks. After dose intensivation in one patient all patients demonstrated improvement by improved radiological images, stressing the importance to adjust dosing in individual patients.

Other potential target cytokines that are involved in the pathogenesis of sarcoidosis are IFN-γ (2) (Figure 1) and IL-12. Both are abundant in serum and are produced by alveolar macrophages from patients with sarcoidosis (47, 48). Overexpression of the pro-inflammatory cytokine IL-12 leads to increased expression of TNF-α and even more IFN-γ, the key cytokines in the formation of granulomas. Therefore, inhibition of IL-12 might have a deteriorating effect on granuloma formation. However, a human monoclonal antibody (anti-P40) that inhibits IL-12 and IL-23 (ustekinumab), does not exert in a clinical beneficial effect in pulmonary sarcoidosis (42). This suggests that IL-12 does not has a major role in the pathogenesis of sarcoidosis, or inhibition of IL-12 does not resolve the disease once granulomas have been formed.

Another possible therapeutic target is IL-6. IL-6 plays a role in the production and maintenance of sarcoid granulomas by activation of CD4+ T cells (49). Tocilizumab is a monoclonal antibody against IL-6 and could be successful in the treatment of sarcoidosis. At the moment tocilizumab is successfully used in patients with rheumatoid arthritis (50). However, thus far no case reports are known in patients with sarcoidosis of this possible effect.

Traditional forms treatment that directly target T cells with drugs such as prednisone or methotrexate might be improved by biological modalities. It could be of interest to produce a new subtype of these drugs that only tar gets the cytokines who are responsible for the disturbed T cell response in sarcoidosis, consequently keeping the risk of side effects as low as possible. As suggested in Chapter 2, also B cells might serve as a therapeutic target, e.g. using rituximab, a chimeric monoclonal anti-CD20 antibody (51).

CD20 is specifically expressed on the surface of naive and memory B cells but not on plasma cells (6). There are a few case reports describing the successful use of rituximab in refractory sarcoidosis with lung, eye, lymph nodes and skin involvement (52-56). However, the only small but prospective study showed inconsistent results (57). It therefore can be concluded that the effectiveness of rituximab in chronic sarcoidosis remains a question and that further trials are necessary to clarify the exact effect of rituximab in sarcoidosis. It is tempting to hypothesize that this may be an additional therapeutic option to other current therapies in sarcoidosis.

It remains a matter of uncertainty how to monitor the efficacy of treatment in sarcoidosis. In chapter 3 and 4 it is shown that SRS may be a tool to monitor efficacy of treatment. Unfortunately this does not concern patients on steroids (first-line therapy). The somatostatin receptor sub type 2 (SST2) is highly expressed by sarcoid granulomas and is used as the target for SRS (58). Treatment with corticosteroids may downregulate SST2 and consequently downgrade uptake of radioactivity in sarcoid granulomas (59). 18F-FDG PET scintigraphy has been shown to be related to sarcoid activity, however this technique has also its limitations, i.e. serum glucose levels and complicated cardiac and brain evaluation (60). The new gallium-DOTA-octreotate imager which is suitable for PET imaging may be a new alternative. However, radionuclide-coupled somatostatin analogues visualize the normal spleen because of an abundant SST2 expression by spleen macrophages and endothelium. Combi imaging with gallium-DOTA-octreotate and 18F-FDG may be theoretically the most sensitive possibility to investigate the extend of sarcoid disease.

Conclusions

In this thesis we revealed the possible pathophysiological role for B cells in granuloma formation in sarcoidosis. We showed that the B cell function is supposed to be normal because the vaccination response is not impaired, ruling out a clinical significant humoral immunodeficiency. We showed that adalimumab is a promising therapy that results in normalization of specific B cell subsets. 25-(OH)D seems to be related to disease activity, supplementation of vitamin D is relative safe and successful in decreasing disease activity. We showed that extend of disease and therapeutic efficacy can effectively be monitored with SRS, a sensitive radio nuclear imaging technique.

Sarcoidosis remains a fascinating disease in which various immune cells and pathways are involved. The complex pathogenesis results in a complex and limited therapeutic approach that needs further

development in order to reduce inflammatory activity, decrease side effects and improve the quality of life of patients with sarcoidosis. This thesis provides new points of action for further research in 'the disease with many faces'.

References

1. Lazarus A. Sarcoidosis: epidemiology, etiology, pathogenesis, and genetics. Dis Mon. 2009;55:649-660.
2. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007;357:2153-2165.
3. Morgenthau AS, Iannuzzi MC. Recent advances in sarcoidosis. Chest. 2011;139:174-182.
4. Baughman RP, Culver DA, Judson MA. A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med. 2011;183:573-581.
5. Lazarus A. Sarcoidosis: epidemiology, etiology, pathogenesis, and genetics. Dis Mon. 2009;55:649-660.
6. Lee NS, Barber L, Akula SM, Siqounas G, Kataria YP, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. Clin Vaccine Immunol. 2011;8:1306-1316.
7. Daniele RP, McMillan LJ, Dauber JH, Rossman MD. Immune complexes in sarcoidosis: a correlation with activity and duration of disease. Chest. 1978;74:261-264.
8. Hunninghake GW, Crystal RG. Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis: site of increased antibody production and role of T lymphocytes. J Clin Invest. 1981;67:86-92.
9. Weinberg I, Vasiliev L, Gotsman I. Anti-dsDNA antibodies in sarcoidosis. Semin Arthritis Rheum. 2000;29:328-331.
10. Fazel SB, Howie SEM, Krajewski AS, Lamb D. B lymphocytes accumulations in human pulmonary sarcoidosis. Thorax. 1992;47:964-967.
11. Fritz JH, Rojas OL, Simard N, McCarthy DD, Hapfelmeier S, Rubino S, Robertson SJ, Larijani M, Gosselin J, Ivanov II, et al. Acquisition of a multifunctional IgA+ plasma cell phenotype in the gut. Nature. 2011;481:199-203.
12. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. Immunology. 2005:115:1.
13. Hijdra D, Vorselaars AD, Grutters JC, Claessen AM, Rijkers GT. Differential expression of TNFR1 (CD120a) and TNFR2 (CD120b) on subpopulations of human monocytes. J Inflamm (Lond). 2012;Oct 5;9(1):38.
14. Saussine A, Tazi A, Feuillet S, Rybojad M, Juillard C, Bergeron A, Dessirier V, Bouhidel F, Janin A, Bensussan A, et al. Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10 producing regulatory B cells and high BAFF levels. PLoS ONE. 2012;7:e43588.
15. Driessen GJ, van Zelm MC, van Hagen PM, Hartwig NG, Trip M, Warris A, de Vries E, Barendregt BH, Pico I, Hop W, et al. B-cell replication history and somatic hypermutation status identify distinct pathophysiological backgrounds in common variable immunodeficiency. Blood. 2011;118:6814-6823.
16. Küppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005;4:251-262.
17. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia: BCR response and IGHV mutation. Nat Rev Cancer. 2010;10:37-50.
18. Papanikolaou IC, Sharma OP. The relationship between sarcoidosis and lymphoma. Eur Respir J. 2010;36:1207-1219.

19. Garcia Casares E, Mateo Soria L, Garcia Melchor E, Minguez Blasco S, Vall-Llobera Calmet F, Canellas Oliver G, Holgado Pérez S. Sarcoidosis-lymphoma syndrome. Reumatol Clin. 2009;1:31-33.
20. Brandy-Garcia AM, Caminal-MonteroL, Fernández-Garcia MS, Saiz Ayala A, Cabezas-Rodríguez I, Morante-Bolado I. Sarcoidosis-lymphoma syndrome. Reumatol Clin. 2015 Dec 16.
21. Chayayer É, Bachy E, Occeli P, Weiler L, Faurie P, Ghesquieres H, Pavic M, Broussolle C, Sève P. Sarcoidosis and lymphoma: a comparative study. QJM. 2015;108:871-878.
22. Karakantza M, Matutes E, McLennan K, O'Connor NT, Srivastava PC, Catovsky D. Association between sarcoidosis and lymphoma revisited. J Clin Pathol. 1996;49:208-212.
23. Artac H, Bozkurt B, Talim B, Reisli I. Sarcoid-like granulomas in common variable immunodeficiency. Rheumatol Int. 2009;30:109-112.
24. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, Vikova M, Hernandez M, Detkova D, Bos PR, et al. The EUROclass trial: defining subgroups in common variable immunodefiency. Blood. 2008;111:77-85.
25. Hermaszewski RA, Webster AD. Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. Q J Med. 1993;86:31-42.
26. Chen H, Liao O, Holl TM, Snowden P, Ueda Y, Kelsoe G. Genetic regulation of pristine-induced oil granuloma responses. Int J Exp Pathol. 2010;91:472-483.
27. Rabin RL, Levinson AI. The nexus between atopic disease and autoimmunity: A review of the epidemiological and mechanistic literature. Clin Exp Immunol. 2008;153:19-30.
28. Bargagli E, Bianchi N, Margollicci M, Olivieri C, Luddi A, Coviello G, Grosso S, Rottolli P. Chitotriosidase and soluble IL-2 receptor: comparison of two markers of sarcoidosis severity. Scand J Clin Lab Invest. 2008;68:479-483.
29. Grutters JC, Fellrath JM, Mulder L, Janssen R, van den Bosch JM, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. Chest. 2003;124:186-195.
30. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-291.
31. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296:2832-2838.
32. Burke RR, Rybicki BA, Rao DS. Calcium and vitamin D in sarcoidosis: how to assess and manage. Semin Respir Crit Care Med. 2010;31:474-484.
33. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet. 2003;361:1111-1118.
34. Hernlund E, Svedbom A, Ivergard M, Compston J, Cooper C, Stenmark E, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden: A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8:136.
35. Majumdar SR, Lix LM, Yogendran M, Morin SN, Metge CJ, Leslie WD. Population-based trends in osteoporosis management after new initiations of long-term systemic glucocorticosteroids (1998-2008). J Clin Endocrinol Metabol. 2012;97:1236-1242.
36. Conron M, Young C, Beynon HL. Calcium metabolism in sarcoidosis and its clinical implications. Rheumatology (Oxford). 2000;39:707-713.
37. Adler RA, Funkhouser HL, Petkov VI, Berger MM. Glucocorticoidinduced osteoporosis in patients with sarcoidosis. Am J Med Sci. 2003;325:1-6.
38. Newman LS, Rose CS, Maier LA. Sarcoidosis. N Engl J Med. 1997;336:1224-1235.

39. Baughman RP, Iannuzzi M. Tumour necrosis factor in sarcoidosis and its potential for targeted therapy. Biodrugs. 2003;17:425-431.
40. Baughman RP, Drent M, Kavuru M, et al. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. Am J Respir Crit Care Med. 2006;174:795-802.
41. Judson MA, Baughman RP, Costabel U, Flavin S, Lo KH, Kavuru MS, Drent M; Centocor T48 Sarcoidosis Investigators. Efficacy of infliximab in extrapulmonary sarcoidosis: results from a randomized trial. Eur Respir J. 2008;31:1189-1196.
42. Judson MA, Baughman RP, Costabel U, Drent M, Gibson KF, Raghu G, Shigemitsu H, Barney JB, Culver DA, Hamzeh NY, Wijsenbeek MS, Albera C, Huizar I, Agarwal P, Brodmerkel C, Watt R, Barnathan ES. Safety and efficacy of ustekinumab or golimumab in patients with chronic sarcoidosis. Eur Respir J. 2014;44:1296-1307.
43. Farah RE, Shay MD. Pulmonary sarcoidosis associated with etanercept therapy. Pharmacotherapy. 2007;27:1446-1448.
44. Khanna D, Liebling MR, Louie JS. Etanercept ameliorates sarcoidosis arthritis and skin disease. J Rheumatol. 2003;30:1864-1867.
45. Tuchinda C, Wong HK. Etanercept for chronic progressive cutaneous sarcoidosis. J Drugs Dermatol. 2006;5:538-540.
46. Utz JP, Limper AH, Kalra S, Specks U, Scott JP, Vuk-Pavlovic Z, Schroeder DR. Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. Chest. 2003;124:177-185.
47. Antoniou KM, Tzouvelekis A, Alexandrakis MG, et al. Upregulation of Th1 cytokine profile (IL-12, IL-18) in bronchoalveolar lavage fluid in patients with pulmonary sarcoidosis. J Interferon Cytokine Res. 2006;26:449-456.
48. Moller DR, Forman JD, Liu MC, et al. Enhanced expression of IL 12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. J Immunol. 1996;156:4952-4960.
49. Urbankowski T, Hoser G, Domagala-Kulawik J. Th1/Th2/Th17 related cytokines in the bronchoalveolar lavage fluid of patients with sarcoidosis: association with smoking. Pol Arch Med Wewn. 2012;122:320-325.
50. Lindegaard HM, Johansen P, Gröndal G, Jensen EC, Juul L, Schlemmer AM, Agular B, Hansen I. Doubling the single-dose infusion rate of tocilizumab in rheumatoid arthritis is safe and efficacious. Scand J Rheumatol. 2016 Jan 4:1-5.
51. Hinze CH, Colbert RA. B-cell depletion in Wegener's granulomatosis. Clin Rev Allergy Immunol. 2008;34:327-379.
52. Gottenberg JE, Guillevin L, Lambotte O, Combe B, Allanore Y, Cantagrel A, Larroche C, Soubrier M, Bouillet L, Dougados M, et al. Club Rheumatismes et Inflammation (CRI). Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases. Ann Rheum Dis. 2005;64:913-920.
53. Belkhou A, Younsi R, El Bouchti I, El Hassani S. Rituximab as a treatment alternative in sarcoidosis. Joint Bone Spine. 2008;75:511-512.
54. Dasilva V, Breuil V, Chevallier P, Euller-Ziegler L. Relapse of severe sarcoidosis with an uncommon peritoneal location after TNF alpha blockade: efficacy of rituximab, report of a single case. Joint Bone Spine. 2010;77:82-83.
55. Bomprezzi R, Pati S, Chansakul C. Vollmer T. A case of neurosarcoidosis successfully treated with rituximab. Neurology. 2010;75:568-570.
56. Cinetto F, Compagno N, Scarpa R, Malipiero G, Agostini C. Rituximab in refractory sarcoidosis: a single centre experience. Clin Mol Allergy. 2015;13:19.
57. Sweiss NJ, Lower EE, Mirsaeidi M, Dudek S, Garcia JG, Perkins D, et al. Rituximab in the treatment of refractory pulmonary sarcoidosis. Eur Respir J. 2014;43:1525-1528.

58. Ten Bokum AM, Hofland LJ, de Jong G, et al. Immunohistological localization of somatostatin receptor SST2A in sarcoid granulomas. Eur J Clin Invest. 1999;29:630-636.
59. De Bruin C, Feelders RA, Waaijers AM, et al. Differential regulation of human dopamine D2 and somatostatin receptor subtype expression by glucocorticoids in vitro. J Mol Endocrinol. 2009;42:47-56.
60. Sobic-Saranovic D, Artiko V, Obradovic V. FDG PET imaging in sarcoidosis. Semin Nucl Med. 2013;43:404-411.
61. Cunningham-Rundles C. Autoimmune manifestations in common variable immunodeficiency. J Clin Immunol. 2008;S42-S45.
62. Ardeniz O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol. 2009;133:198-207.

Chapter 6

Summary
Samenvatting

Summary

Sarcoidosis is a granulomatous disorder of unknown cause affecting multiple organs and is characterized by non-caseating granulomas. Usually sarcoidosis develops before the age of 50 years, with an incidence peaking at 20 to 39 years. The worldwide prevalence varies from 2 to 80 per 100,000. In the Netherlands the prevalence is estimated to be 50 per 100,000. However, in 30-60% of the cases the prevalence may be underestimated by the asymptomatic signs of the disease.

Although sarcoidosis was first described in 1877, the pathophysiology and what triggers the disease remains still a question. It is presumed that granulomas play a central role in the pathogenesis of sarcoidosis. The hypothesis in sarcoidosis is that granulomas are formed by an interaction between antigen presenting cells and activated T-lymphocytes, mainly CD4+ T cells. This leads to a release of cytokines which triggers macrophages to produce TNF-α and strengthen the local inflammation response by releasing a cascade of several cytokines. In this thesis aspects of sarcoidosis on areas pathophysiology, diagnostics and new treatment modalities will be highlighted.

In **Chapter 2** we revealed the possible pathophysiological role for B cells in granuloma formation in sarcoidosis. We observed increased circulating transitional B cell numbers and increased molecular signs of antibody maturation, which indicates presence of an overactive B cell response. Thus therapy that targets B cells might serve as a therapeutic option. Similar to CVID patients (an antibody deficiency disorder) we demonstrated reduced numbers of blood memory B cells in sarcoid patients. But in contrast to CVID patients with granulomas, patients with sarcoidosis have a normal vaccination response, making it unlikely that patients with sarcoidosis have impaired B cell responses. Furthermore, we demonstrated that treatment with a TNF-α blocker normalizes the population of CD27-IgA+ memory B cells that we found reduced in untreated patients. Thus, blood CD27-IgA+ memory B cell numbers could be a potential marker of sarcoidosis activity.

Sarcoidosis is thought to be a mainly Th-1 driven disease. We confirmed this by demonstrating the lack of association between atopy (a Th-2 driven disease) and sarcoidosis.

Traditionally, sarcoidosis is diagnosed by compatible clinical and conventional radiological findings (X-ray and CT scan), the presence of non-caseating granulomas and exclusion of similarly presenting disorders. In **Chapter 3** we showed that the SRS has an added value in the diagnostic workup and is more sensitive than conventional imaging in sarcoidosis

patients. SRS revealed significantly more thoracic lesions than conventional radiologic imaging. Our observations hence indicate that the extend of sarcoid disease remains underestimated with conventional imaging and underscore the assumption that negative conventional imaging for sarcoidosis does not exclude pulmonary involvement.

Hypercalcemia is described in 5-11% of the sarcoidosis patients. Therefore, calcium and vitamin D supplementation is not without risk. Nevertheless we demonstrated in **Chapter 4** that supplementation of calcium and vitamin D is relatively safe in sarcoidosis patients if there is no hypercalcemia at moment of starting the supplementation. We also described an inverse relation between disease activity of sarcoidosis measured by SRS and serum levels of 25-(OH)D. If indeed decreased levels of serum 25-(OH)D are a potential risk factor for higher disease activity in sarcoidosis patients supplementation of vitamin D might not only be of prophylactic (in case of high dosages of corticosteroids), but also of therapeutic value.

Finally we present data of a case series of five patients with symptomatic, chronic, both pulmonary and extra pulmonary sarcoidosis treated with adalimumab (TNFα-blocker). Clinical and radiological improvement occurred within 12 weeks in four patients. After dose intensivation in one patient all patients demonstrated improvement. This stresses the importance to adjust dosing of TNFα-blockers in individual patients. **Chapter 5** contains a general discussion in which the findings described in this thesis are put into a broader perspective. In conclusion, the studies described in this thesis provided new insights in the complex pathophysiology in sarcoidosis and thereby provide possibly new parameters for disease activity, diagnostics and targets for treatment.

Samenvatting

Sarcoïdose is een multisysteemziekte van onbekende oorzaak en wordt gekarakteriseerd door niet-necrotiserende granulomen. Sarcoïdose wordt meest al gediagnosticeerd vóór het 50e levensjaar met een incidentiepiek tussen de 20 en 39 jaar. De prevalentie varieert wereldwijd van 2-80 patiënten per 100.000 inwoners. In Nederland wordt de incidentie geschat op 20 per 100.000 per jaar en de prevalentie op 50 per 100.000. Dit is waarschijnlijk een onderschatting, omdat de ziekte vaak (bij 30-60% van de patiënten) asymptomatisch verloopt.

Sarcoïdose werd voor het eerst in 1877 beschreven, echter is de pathofysiologie nog steeds niet duidelijk. Aangenomen wordt dat granulomen een centrale rol spelen in de pathofysiologie sarcoïdose. De hypothese is dat bij sarcoïdose granulomen gevormd worden door interactie tussen antigeen-presenterende cellen en geactiveerde T-lymfocyten, voornamelijk CD4+ T-cellen. De hierbij vrijgekomen cytokinen zetten macrofagen aan tot productie van TNF-α en versterken de lokale ontstekingsrespons door het vrijkomen van een cascade van verschillende cytokinen. In dit proefschrift wordt de pathofysiologie, de diagnostische technieken en nieuwe behandelopties bij sarcoïdose besproken.

In **hoofdstuk 2** laten we de mogelijke pathofysiologische rol van de B-cel bij granuloomvorming zien in sarcoïdose patiënten. We hebben ook aan getoond dat er in sarcoïdose sprake is van een verhoogd aantal circulerende transitionele B-cellen en dat de uitrijping van antistoffen verhoogd is. Dit wijst op betrokkenheid van een overactieve B-cel respons in de pathofysiologie van sarcoïdose met als gevolg dat de B-cel een goed aangrijpingspunt zou zijn voor behandelopties. Patiënten met CVID (een antistofdeficiëntie) hebben een verlaagd aantal geheugen B-cellen in het perifere bloed wat gelijk is aan onze bevindingen bij sarcoïdose patiënten. Echter in tegenstelling tot een subgroep van CVID patiënten (patiënten met granulomateuze CVID), laten sarcoïdose patiënten wel een normale vaccinatierespons zien. Dit maakt het onwaarschijnlijk dat patiënten met sarcoïdose een gestoorde B-cel respons hebben. We hebben tevens aangetoond dat na behandeling met een TNF-α remmer het aantal CD27-IgA+ geheugen B-cellen normaliseert. Dit suggereert dat CD27-IgA+ geheugen B-cellen een mogelijke marker zouden kunnen zijn voor ziekte-activiteit.

Van sarcoïdose wordt gezegd dat het voornamelijk een Th-1 ziekte is. Dit hebben wij bevestigd door de afwezigheid van een associatie tussen atopie (een Th-2 gemedieerde ziekte) en sarcoïdose.

Sarcoïdose wordt gediagnosticeerd indien er sprake is van klinische verschijnselen die compatibel zijn met de ziekte, radiologische

afwijkingen (op een thoraxfoto en/of CT-scan) en de aanwezigheid van niet-necrotiserende granulomen. Echter dienen andere ziektes wel uitgesloten te zijn. In **hoofdstuk 3** tonen we aan dat de SMS-scan een toegevoegde waarde heeft in de diagnostische work-up bij sarcoïdose. Tevens laten we zien dat deze techniek sensitiever is dan conventionele beeldvorming in patiënten met sarcoïdose. De SMS-scan toont significant meer pulmonale laesies aan vergeleken met conventionele radiologische beeldvorming. Onze observaties suggereren daarom dat de uitgebreidheid van de ziekte onderschat wordt met alleen conventionele beeldvorming. Daarnaast betekent een niet-afwijkende thoraxfoto en/of CT-scan niet dat er geen pulmonale betrokkenheid van de sarcoïdose bestaat.

Hypercalciëmie wordt bij 5 tot 11% van de patiënten met sarcoïdose beschreven. Daarom is calcium en vitamine D suppletie niet zonder risico. Echter tonen wij aan in **hoofdstuk 4** dat calcium en vitamine D suppletie wel relatief veilig gegeven kan worden, mits er op moment van starten geen sprake is van een hypercalciëmie. Daarnaast beschrijven we een omgekeerde relatie tussen ziekte-activiteit van sarcoïdose gemeten met de SMS-scan en serum waardes van 25-(OH)D. Indien verlaagde waardes van 25-(OH)D inderdaad een potentiële risicofactor zijn voor meer ziekte-activiteit in sarcoïdose, dan heeft vitamine D suppletie niet alleen een profylactische waarde (bij corticosteroïden gebruik), maar ook therapeutisch.

Tenslotte presenteren we data van een case serie van vijf patiënten met symptomatische, chronische en zowel pulmonale als niet-pulmonale betrokkenheid van de sarcoïdose die behandeld worden met adalimumab (TNF-α remmer). Klinische en radiologische verbetering was zichtbaar na 12 weken behandeling bij vier van de vijf patiënten. Na het intensiveren van de adalimumab behandeling toonde ook de laatste patiënt verbetering. Dit benadrukt het belang dat de dosering van TNF-α remmers op de individu aangepast moet worden.

Hoofdstuk 5 bevat de algemene discussie waarin de resultaten die beschreven zijn in dit proefschrift in een breder perspectief worden geplaatst. Concluderend beschrijven onze studies nieuwe inzichten in de complexe pathofysiologie van sarcoïdose, nieuwe diagnostische opties en nieuwe aangrijpingspunten voor behandeling.

Chapter 7

Dankwoord
Curriculum Vitae
List of Publications
PhD Portfolio

Dankwoord

En dan ben ik eindelijk toegekomen aan het meest gelezen gedeelte van een proefschrift. De afgelopen jaren heb ik met veel plezier aan dit proefschrift gewerkt. Uiteraard is dit boekje tot stand gekomen met de hulp van anderen, wie ik met dit dankwoord wil bedanken.

Beste Martin. In 2008 leerde ik je kennen als docent van het immunologie onderwijs en ik was meteen onder de indruk van je enthousiasme, maar met name van al je kennis. Niet veel later ging ik in jouw groep aan de slag als onderzoekster en dat als niet-Aziaat. Heel erg bedankt voor al je raad en wijze adviezen, ook buiten het werk om. Ik heb altijd erg genoten van onze congressen samen. Dat spookhotel in San Antonio zal ik nooit meer vergeten!

Beste Jan, mijn co-promotor. Ik weet nog goed dat jij mij aan het begin van de nacht belde om te vertellen dat ons eerste artikel geaccepteerd was. Jouw enthousiasme motiveerde mij om van het volgende artikel ook weer een topartikel te maken. En dat lukte! Heel erg bedankt voor je hulp de afgelopen jaren.

Beste Menno. Opeens kreeg je er een promovenda uit de kliniek bij. Ik ben vereerd dat jij mijn co-promotor wilde zijn. Jouw kennis en deename aan de klinische onderzoeken heb ik altijd erg gewaardeerd. Jouw aanvullingen waren zeer waardevol en tilden de artikelen en ook dit proefschrift naar een hoger niveau. Veel succes in Melbourne.

Paul, Virgil, Jasper, Kiki, Marianne, Kim, Leendert, Jeroen en Wim, dank voor jullie steun, gezelligheid en de leuke congressen die we samen hebben gehad.

Benjamin en Edwin heel erg bedankt voor al jullie hulp bij de analyses op het lab. Edwin jij was in begin mijn grote steun op het lab, ik kon echt alles aan je vragen en je was altijd bereid om mij te helpen en mee te denken. Benjamin, jij nam later de taak van Edwin over, ik waardeer het zeer dat je zelfs je vrije dagen opgaf om mij te kunnen helpen bij de analyses. Het is gelukkig niet voor niets geweest!

Beste Judy, ook jij heel erg bedankt voor je hulp (met name met al het papierwerk). Het jaar dat we samen een kamer deelden op de D-vleugel heb ik als een erg leuke tijd ervaren en ons reisje naar New York was natuurlijk helemaal onvergetelijk!

Femke en WaiKwan, ook jullie wil ik bedanken. Jullie namen mij als 3e jaars geneeskunde studente onder jullie hoede en hebben mij enthousiast gemaakt om door te gaan met wetenschappelijk onderzoek, zie hier het eindresultaat!

Beste professor Hoogsteden, bedankt voor de opleidingsplek tot longarts die je mij aanbood. Ik kijk uit naar het verdere vervolg van deze opleiding op jouw afdeling.

Dr. van den Toorn, mijn opleider in het Erasmus MC en dr. Rietveld, mijn oud-opleider in het Sint Franciscus Gasthuis, ook jullie wil ik bedanken voor de steun en begrip de afgelopen jaren. Ik wil al mijn (oud) collega's uit Sint Franciscus Gasthuis en het Erasmus MC bedanken voor de steun tijdens deze promotie.

Eva, Laura, Chang, Stefanie en Dorien, heel erg bedankt voor jullie motiverende gesprekken en goede adviezen als ik het even niet meer zag zitten. Ik hoop dat we nog veel leuke dingen samen gaan doen. En Dorien misschien komt er ooit nog wel een doliboka!

Joost, Anja en Nieke ook jullie wil ik bedanken. Bedankt voor de steun bij de afronding van het proefschrift en zeker de inspiratie voor de omslag! Dat we samen nog veel gezellige dagen gaan beleven.

Susan! Wat ben ik blij dat je vandaag naast me staat als paranimf.

Lieve Oma, ik zeg al een hele tijd tegen jou dat ik binnenkort ga promoveren en dat je daar absoluut bij moet zijn. Het is een paar keer spannend geweest of dat voor jou nog wel zou lukken, maar gelukkig is het nu zo ver. Ik ben blij dat ik deze dag samen met jou mee mag maken!

Papa en mama, ook al is het soms niet makkelijk dat we niet dicht bij elkaar wonen, maar jullie staan altijd voor me klaar en hebben me gesteund in al mijn keuzes. Dank jullie wel daarvoor! Ik ben trots dat ik jullie dochter ben. Brian, ondanks dat jij zelf moeilijke jaren hebt doorgemaakt en we elkaar niet vaak zien, heb je mij altijd gesteund en maak je mij weer aan het lachen (of ik jou…). Ik ben dan ook erg trots dat jij mijn paranimf bent vandaag!

Lieve Sander, bedankt voor je steun en dat je er altijd voor me bent. Jouw liefde en humor zijn van onschatbare waarde geweest. Ik kijk uit naar onze toekomst samen. Ik hou van jou!!

Curriculum Vitae

Lieke Kamphuis werd op 8 juni 1985 geboren in Oldenzaal. Zij volgde het voortgezet wetenschappelijk onderwijs aan het Twents Carmel College te Oldenzaal. In 2004 werd zij toegelaten voor de studie geneeskunde aan de Erasmus Universiteit in Rotterdam. In 2008 begon ze, naast haar studie geneeskunde, te werken bij de klinische immunologie onder begeleiding van Prof. dr. P.M. van Hagen en Dr. J.A.M. van Laar. Dit resulteerde in een promotie onderzoek op deze afdeling naar de ziekte sarcoïdose. In 2012 behaalde Lieke cum laude haar artsexamen.

Gedurende haar promotietraject heeft zij meerdere prijzen gewonnen, waaronder 2 travel awards, best clinical paper van de SNM Young Professionals Committee, beste presentatie bij de internistendagen en was ze winnaar van de onderzoeksprijs van de sarcoïdose belangenvereniging in Nederland.

Per 1 januari 2013 is Lieke begonnen met de opleiding Longziekten en Tuberculose (opleiders Dr. L. van den Toorn en Dr. A. Rietveld) in het Erasmus Medisch Centrum en Sint Franciscus Gasthuis in Rotterdam.

List of publications

- Sarcoïdose en de Mexicaanse griep.
  Kamphuis LSJ, van Biezen P, Osterhaus ADME, van der Eijk AA, van Laar JAM, van Daele PLA, van Hagen PM.
  Sarcoïdose Nieuws. September 2009.

- Detectie van cytokinen in de diagnostiek van ontstekingsprocessen.
  Dik WA, van Laar JAM, Smits-te Nijenhuis M, Kolijn-Couwenberg MJ, Nagtzaam NMA, Bastiaans J, Kamphuis L, Kuijpers RWAM, van Hagen PM, Hooijkaas H.
  Nieuwe ontwikkelingen in de Medische Immunologie 2010.
  ISBN 978-90-73436-91.

- Sarcoïdose.
  Kamphuis LSJ, van Hagen PM.
  Sarcoïdose Nieuws. Juni 2010.

- Sarcoidosis: changing insights in therapy.
  Kamphuis LS, van Laar JA, Kuijpers RW, Missotten T, Thio HB, van Hagen PM.
  Ned Tijdschr Geneeskd. 2010;154:A1685.

- De werking van biologicals bij sarcoïdose.
  Kamphuis LSJ, Wijsenbeek M, van Hagen PM.
  SarcoScoop. September 2011.

- Efficacy of adalimumab in chronically active and symptomatic patients with sarcoidosis.
  Kamphuis LS, Lam-Tse WK, Dik WA, van Daele PL, van Biezen P, Kwekkeboom DJ, Kuijpers RW, Hooijkaas H, van Laar JA, Bastiaans J, Baarsma GS, van Hagen PM.
  Am J Respir Crit Care Med. 2011 ;184:1214-1216.

- Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis?
  Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma GS, Dik WA, Thio HB, van Daele PL, van Velthoven ME, Batstra MR, van Hagen PM, van Laar JA.
  Am J Respir Crit Care Med. 2013;187:406-416.

- Calcium and vitamin D in sarcoidosis: is supplementation safe?
  Kamphuis LS, Bonte-Mineur F, van Laar JA, van Hagen PM, van Daele PL.
  J Bone Miner Res. 2014;29:2498-2503.

- Prevalence of atopic disease in patients with sarcoidosis.
  Hajdarbegovic E, Kamphuis L, van Laar J, van Hagen M, Nijsten T, Thio B.
  Allergy Asthma Proc. 2014;35:57-61.

- Somatostatin receptor scintigraphy in patients with sarcoidosis.
  Kamphuis LS, van Hagen PM, Baarsma GS, Dalm VA, van Laar JA, Dik WA, Timmermans WM, Kwekkeboom DJ.
  Clin Nucl Med. 2015;40:925-929.

- Granulomatous common variable immunodeficiency; the extend of disease.
  Kamphuis LS, Kwekkeboom DJ, Driessen GJ, van der Burg M, Atmaca S, Misotten T, van Laar JA, Dalm VA, van Hagen PM.
  Submitted.

- B-cell dysregulation in Crohn's disease is partially restored with infliximab therapy.
  Timmermans WM, van Laar JA, van der Houwen TB, Kamphuis LS, Bartol SJ, Lam KH, Ouwendijk RJ, van Hagen PM, van Zelm MC.
  Submitted.

PhD Portfolio

Name PhD student:	L.S.J. (Lieke) Kamphuis
Erasmus MC Department:	Internal Medicine, section Clinical Immunology & Immunology
Promotor:	Prof. dr. P.M. van Hagen
Co-promotors:	Dr. J.A.M. van Laar Dr. M.C. van Zelm

General courses

2011	CPO minicursus (Methodologie van patiëntgebonden onderzoek en voorbereiding van subsidie aanvragen)

Oral presentations

2010	Science days Department of Internal Medicine. B cell development in sarcoidosis. Antwerp, Belgium.
2011	Dutch Internal Medicine Days. Somatostatin receptor scintigraphy in sarcoidosis patients. Maastricht, The Netherlands.
2011	Dutch Internal Medicine Days. Efficacy of adalimumab in sarcoidosis. Maastricht, The Netherlands.
2011	Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging. Somatostatin receptor scintigraphy in sarcoidosis patients. San Antonia, USA.
2011	Meeting of patients with Primary Immunodefiencies. Granulomas in CVID. Rotterdam, The Netherlands.
2012	Annual Meeting Sarcoïdose Belangenvereniging. New immunologic, diagnostics and treatment insights in sarcoidosis. Wageningen, The Netherlands.

2012	Dutch Internal Medicine Days. Are B cells emerging as key players in the pathogenesis of sarcoidosis? Maastricht, The Netherlands.
2012	Annual Meeting of State of the Art in Uveitis. Sarcoïdose: een overzicht van de huidge stand van zaken. Schiermonnikoog, The Netherlands.
2012	Meeting about Sarcoidosis & Idiopathic Pulmonary Fibrosis. Nieuwe visualisatie technieken van sarcoïdose. Rotterdam, The Netherlands.
2013	Meeting Sarcoïdose Belangenvereniging. Een update over de nieuwste scans en wat doet vitamine D met de ziekte sarcoïdose? Bergen op Zoom, The Netherlands.

Poster presentations

2009	European Workshop on Immune-Mediated Inflammatory Diseases. B cell development in sarcoidosis. Lisbon, Portugal.
2010	Biennial Meeting European Society for Immunodeficiencies. B cell development in granulomatous diseases. Istanbul, Turkey.
2010	European Workshop on Immune-Mediated Inflammatory Diseases. Efficacy of adalimumab in sarcoidosis. Sitges, Spain.
2011	Science days Department of Internal Medicine. Efficacy of adalimumab in sarcoidosis. Antwerp, Belgium.

2011	Meeting of World Associaton of Sarcoidosis and Other Granulomatous Disorders. Are B cells emerging as key players in the pathogenesis of sarcoidosis? Maastricht, The Netherlands.
2011	Meeting of World Associaton of Sarcoidosis and Other Granulomatous Disorders. Somatostatin receptor scintigraphy in sarcoidosis. Maastricht, The Netherlands.
2011	Meeting of World Associaton of Sarcoidosis and Other Granulomatous Disorders. Efficacy of adalimumab in sarcoidosis. Maastricht, The Netherlands.
2011	Meeting of World Associaton of Sarcoidosis and Other Granulomatous Disorders. Calcium and vitamin D suppletion in sarcoidosis. Maastricht, The Netherlands.
2011	European Workshop on Immune-Mediated Inflammatory Diseases. Somatostatin receptor scintigraphy in sarcoidosis. Nice, France.
2012	Science days Department of Internal Medicine. Somatostatin receptor scintigraphy in sarcoidosis. Antwerp, Belgium.
2012	Annual Meeting of the Association for Research in Vision and Ophthalmology. Somatostatin receptor scintigraphy in ocular sarcoidosis. Fort Lauderdale, USA.
2012	Biennial Meeting European Society for Immunodeficiencies. Granulomatous disease visualized by somatostatin receptor scintigraphy in common variable immunodeficiency. Florence, Italy.

2014	Biennial Meeting European Society for Immunodeficiencies. Granulomatous disease in common variable immunodeficiency. Prague, Czech Republic.

Lecturing

2010	Presentation for 3rd year Medical Students about sarcoidosis.
2011	Presentation for 2nd year Medical Students about sarcoidosis.
2011	Presentation for Master Students of Infection & Immunity about immunological disorders.
2012	Presentation for Master Students of Infection & Immunity about sarcoidosis.
2012	Presentation for 2nd year Medical Students about immunological disorders.
2012	Presentation for 2nd year Medical Students about sarcoidosis.

Awards

2010	Travel Award of the European Workshop on Immune-Mediated Inflammatory Diseases.
2011	Sarcoïdose Belangenvereniging. Winner of the investigator award.
2011	Dutch Internal Medicine Days. Winner of the best oral presentation.
2011	Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging. 2nd place as Best Clinical Paper from the SNM Young Professionals Committee.
2011	Travel Award of the European Workshop on Immune-Mediated Inflammatory Diseases.