Chronic post-inflammatory fatigue in sarcoidosis

from cytokines to behavior

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Chronische post-inflammatoire vermoeidheid na sarcoïdose
(met een samenvatting in het Nederlands)

Proefschrift

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“There is nothing is either good or bad
but thinking makes it so.”
(Shakespeare)

Contents

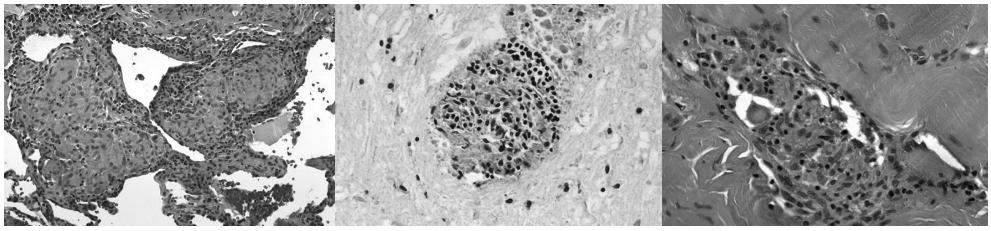
1

General introduction

Sarcoidosis patients frequently report fatigue. Even when sarcoidosis is clinically in remission, fatigue may persist to become a chronic complaint. This type of chronic fatigue has once been referred to as post-sarcoidosis chronic fatigue syndrome: "*a complex of clinical features as incapacitating fatigue, low-spiritedness, wide-spread myalgia and sleep disturbances*."[1] At outpatient clinics, doctors are frequently confronted with sarcoidosis patients who clinically show no signs of disease activity anymore but still suffer from chronic and disabling fatigue. These complaints often lead to a severe reduction in quality of life and to labor disputes with respect to inability to work.
Although this chronic fatigue is clinically and scientifically acknowledged as a serious problem, this thesis is the first to determine the exact features and severity of fatigue in case of clinical remission of sarcoidosis.

Moreover, this thesis will focus on psycho-biological factors that could be associated with this chronic post-inflammatory fatigue in sarcoidosis patients. We will include four factors: cytokine/chemokine profiles; the neuroendocrine system; the nociception by peripheral nerve fibers; and personality and psychological factors.
In the next paragraphs these factors will be clarified, after introducing the two main characters of this thesis first: sarcoidosis and chronic fatigue.

Figure 1 Granuloma's in interstitium, brain, and muscle tissue



Figures are obtained by the Atlas of granulomatous diseases, Y. Rosen, http://granuloma.homestead.com

Sarcoidosis

Sarcoidosis is a systemic inflammatory disorder that is characterized by granuloma formation in different organs. The diagnosis of the disease is established when clinical-radiological findings are supported by histological evidence of noncaseating epitheloid cell granulomas, and granulomatous diseases of known causes an local sarcoid reactions have been excluded.[2]

Pathogenesis

Central to the pathogenesis of sarcoidosis is the activation of naïve CD4+ T cells that differentiate into type 1 helper (Th1) cells. These Th1 cells secrete predominantly interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α. Subsequently, IFN-γ stimulates macrophages to produce TNF-α and IFN-γ, thus creating a cytokine-loop. The complex of this immune response leads to the development and accumulation of granulomas which is the hallmark of the disease. Granuloma formation may be found in any organ, but most often involves the lymphatic system and lungs.[2-4]

Incidence, course and prognosis

Sarcoidosis affects people throughout the world. The incidence and course of the disease varies with ethnicity.[2] Although clear cut epidemiologic data are not available, the prevalence of sarcoidosis in the Netherlands is estimated at approximately 40 per 100.000 individuals. Incidence rates may vary between 10-20 per 100.000 per year.[5] Sarcoidosis affects men and women equally, predominantly in the age of 20 to 40 years. Generally the disease resolves in the majority of patients within 2 to 4 years. In 20 to 40% of the patients sarcoidosis induces fibrosis in the lungs and/or other organs.[2]

Therapy

Systemic corticosteroids are the first-choice therapy in organ- and/or life-threatening sarcoidosis, for example when eyes, heart and central nervous system are affected.[6] Although corticosteroids and other immunosuppressive or immunomodulatory drugs can be clearly effective in sarcoidosis, these drugs do not represent curative treatment, as relapses frequently occur after tapering of these drugs.

Disease activity

There are no internationally accepted criteria for a classification of ‘active disease’ and ‘clinical remission’ in sarcoidosis. Therefore, the criteria for clinical remission of sarcoidosis which we will use in this thesis are based on the available literature[7;8] and clinical experience (JvdB en JG). Principally, these criteria cover different levels of investigation:

1) physical examination,
2) serological tests (ACE, sIL-2R, Ca^{2+}, CRP),
3) imaging (chest X-ray), and
4) functional testing of the lungs.

In addition, patients have to be free of use of corticosteroids or other immunosuppressive drugs over the past 6 months. Moreover, we mention here that only patients with histologically proven sarcoidosis are included, and that patients diagnosed with the acute onset (also known as Löfgren’s syndrome) are excluded from participation in our study because this type of sarcoidosis is known to resolve spontaneously and uncomplicated.

Table 1 Criteria for clinical remission of sarcoidosis as used in this thesis

Physical examination of previously involved organs	no clinical signs of disease activity
Serum parameters (sIL-2R, ACE, Ca^{2+}, CRP)	normal
Chest X-ray	normal or 2 years or more stable
Lung function tests	normal or 2 years or more stable, i.e.:
VC or FEV_1	<10% change
DLco	<15% change

sIL-2R = soluble interleukin-2 receptor, ACE = angiotensin converting enzyme, Ca^{2+}= calcium, CRP = C-reactive protein, VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity for carbon monoxide

Chronic fatigue

Chronic fatigue is defined as "*a self-reported reduction of physical and/or mental well-being which persists more than 6 months and is manifested as exhaustion by which one fails to function at the desired level.*"[9]

Fatigue and diseases

Fatigue is a subjective and unspecific (but important) characteristic of many illnesses and diseases. To define chronic fatigue it is essential to exclude medical conditions that can explain fatigue.

According to the usual clinical work-up to diagnose chronic fatigue, evaluation in our study involved[10;11]:

1) laboratory tests:

Hb, blood cell count, sodium and potassium, creatinine, GGT, AF, ASAT, ALAT, LDH, glucose, total protein, TSH, ferritine, routine urine analysis

2) anamnesis to exclude:

sleep apnea, narcolepsy or restless legs syndrome, psychiatric diseases, and abuse of alcohol, drugs or other substances

3) determination of Body Mass Index (BMI) (kg/m^2)

Chronic fatigue after inflammatory diseases

Chronic post-inflammatory fatigue is a well-known debilitating symptom that may persist even when an infection or inflammatory process has resided. The literature gives ample evidence of chronic fatigue syndrome after infections with viruses like Epstein-Barr virus[12], Ross River virus[13], Coxiella burnetii[14;15], and enterovirus.[16;17] But also inflammatory diseases have been associated with chronic fatigue such as rheumatic arthritis[18], cancer[19;20] or Crohn's disease.[21]

From all these reports it is obvious that, irrespective of the type of infection or inflammation, patients report – besides fatigue – the same constellation of fatigue-related symptoms: pain, muscular weakness, poor mental concentration, and increased and long-lasting malaise after exertion.

Chronic fatigue in sarcoidosis in clinical remission

As already stated in the previous paragraph, chronic fatigue frequently persists after clinical remission of sarcoidosis. However, to the best of our knowledge no studies have systematically investigated this particular problem yet. The exact features and severity of this post-inflammatory fatigue are unknown as well as the underlying psycho-biological factors that maintain the symptoms of chronic post-inflammatory fatigue. As a consequence, adequate management strategies for chronically fatigued sarcoidosis patients in clinical remission are lacking. Management of chronic fatigue is therefore often based on empirical basis, ranging from a trial with low dose corticosteroids or antidepressive medication to physical training programs, psychotherapy, diet and lifestyle advices, or instructions to rest.[22;23] In addition, recently some pilot studies have reported successful treatment with the drug dexmethylphenidate, a norepinephrine-dopamine reuptake inhibitor.[24]

Measurement of chronic fatigue

Currently, there is no laboratory test, brain scan or other objective measurement available to assess fatigue. However, there are more than 252 questionnaires available to assess this subjective symptom.[25] This wide range of questionnaires can be subdivided in ad hoc or one-item questions, multisystem scales, disease-specific scales and fatigue-specific scales.
In the Netherlands, the Checklist Individual Strength (CIS)[26] is one of the most frequently used questionnaires to measure fatigue. We preferred the CIS because this is a fatigue-specific questionnaire. Moreover, the CIS possesses excellent psychometric properties concerning internal reliability and validity, and is widely used to measure fatigue in different diseases as well as in healthy individuals. In this way, the use of the CIS enabled us to compare fatigue scores of sarcoidosis patients with scores on chronic fatigue in other diseases and illnesses. In addition, we used a multi-symptom scale (Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36))[27;28] in order to evaluate general health status as well.

Cytokines and chemokines

In search of factors that could be associated with chronic post-inflammatory fatigue in sarcoidosis patients, we will firstly focus on the immune system, since sarcoidosis is an immune-mediated inflammatory disease. To investigate subclinical immune activity, we

choose to determine in vitro cytokine/chemokine production and plasma cytokine/chemokine profiles in patients with sarcoidosis in clinical remission.

Cytokines and chemokines comprise a heterogeneous group of soluble proteins that are produced by many cell types in the body including immunocompetent cells, such as lymphocytes and macrophages, in order to regulate immune responses.[29] Cytokines/chemokines provide communication between components of the immune system (for example in case of acute phase reaction of a systemic inflammation), and between the immune system and other organ systems, like the brain and the peripheral nervous system. Chemokines are specifically known as mediators that attract immune cells (such as granulocytes, macrophages, T cells) to the site of inflammation.
Cytokines and chemokines (and their receptors) can be classified in families according their structure and/or function, or genetic affiliation. A functional classification is that of T helper cell (Th) 1 and Th2 cytokines.[29] The term Th1 cytokines refers to cytokines that are produced by Th1 cells, and include IL-2, IFN-γ, TNF-α, and IL-12. Th2 cytokines are those produced by Th2 cells, for example IL-4, IL-5, IL-6, IL-10 and IL-13. However, it should be mentioned that the production of most of these cytokines is not limited to the lymphocytes. These are produced by other cell types such as macrophages, liver cells, and endothelial cells as well.

Cytokines in sarcoidosis

Cytokines play an important role in the pathogenesis of sarcoidosis. The initial phase of the disease is dominated by a profound Th1 cytokine profile. Increased levels of IL-2, IFN-γ and TNF-α produced by Th1 cells and activated macrophages are observed in the alveolar compartment as well as in peripheral blood.[30] The subsequent phase of sarcoidosis, after granuloma formation, is characterized by a skewing to a Th2 cytokine production of the T cells in the alveoli.[31] This shift from a Th1 to a Th2 dominated pattern of the T cells in the lung has been proposed to restore the Th1/Th2-balance. Nevertheless, an overshoot to a Th2 pattern has been associated with the chronic form of sarcoidosis and the development of fibrosis in lungs and/or other organs.[30;31]

Cytokine-induced sickness behavior

Apart from the primary function of immune regulation, cytokines have been shown to be associated with the existence of non-specific symptoms of disease also known as cytokine-induced sickness behavior.[32-34] Sickness behavior includes fatigue, general malaise, depressed mood, pain and reduced appetite. This constellation of psychological and behavioral factors represent, together with the fever response and the associated neuroendocrine changes, a highly organized strategy of the organism to fight infection.[35;36] The mechanisms that mediate sickness behavior are studied in the past decades. It is now acknowledged that cytokines

like IL-1 play a key role in the induction of sickness behavior. In animal studies peripheral administration of a cytokine inducer like lipopolysaccharide (LPS), or of a recombinant cytokine such as IL-1β induces symptoms of sickness behavior: fever, loss of appetite, immobility, and behavioral depression. Conversely, administration of cytokine antagonists like IL-10 and IL-4 inhibits the physiologic and behavioral effects of LPS.[37;38] Although IL-1β certainly plays a major role, it is not the sole cytokine that mediates sickness behavior. Also IL-6, IFN-γ and TNF-α may interact in the cytokine network that is associated with sickness behavior.[39;40]
Besides experiments in animals, sickness behavior has first been observed in humans during administration of inflammatory cytokines as treatment of disease. In chronic hepatitis C patients[41-44] as well as in patients with malignant melanoma[45], IFN-α therapy induced severe alterations in mood. Moreover, treatment with TNF-α blockers in rheumatic arthritis not only decreased disease activity but also improved depressive symptoms[46;47]
In addition, sickness behavior in humans naturally occurs after acute infection, such as Epstein-Barr virus, Q-fever or Ross River virus. Cytokines as IL-1β and IL-6 in sera and supernatants of peripheral blood mononuclear cell cultures appeared to be consistently correlated with fever, malaise, pain, fatigue, mood and poor concentration.[48]

In this thesis we acknowledge the association of cytokines and behavior. Therefore, we will determine whether a dysbalance in cytokine/chemokine patterns is associated with post-inflammatory fatigue in these sarcoidosis patients.

Neuroendocrine system: HPA axis and sympathetic nevous system

The second focus in our search of factors that might be associated with chronic post-inflammatory fatigue in sarcoidosis is a possible dysfunction of the neuroendocrine system, particularly in response to a stressor. It is now well established that there is a bi-directional communication between the immune system and the hypothalamic pituitary adrenal axis (HPA axis) as well as the sympathetic nervous system (SNS).[49] The functioning of the HPA axis and the SNS might therefore be a factor of importance when studying post-inflammatory fatigue in immune-mediated diseases such as sarcoidosis.

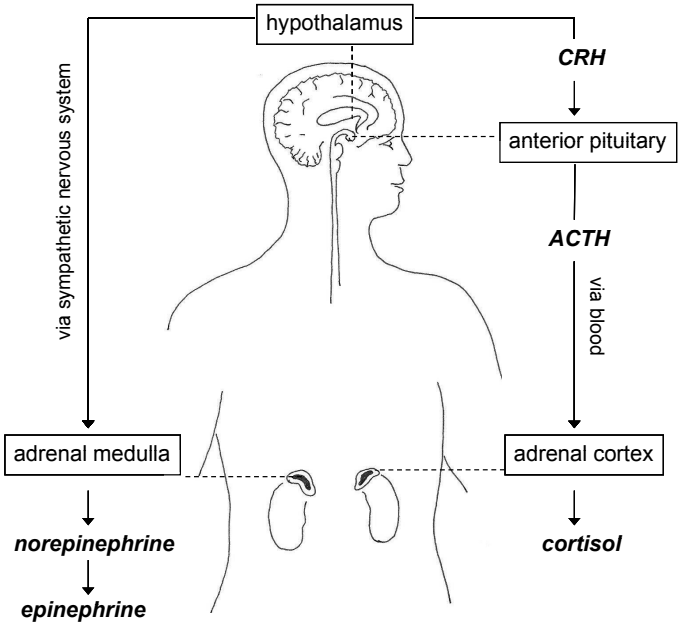
The HPA axis and the SNS system are both involved in the cascade of adaptive responses that is launched in response to acute stress.[50] Under stressful conditions, the body is instantly activated, also known as the ‘flight-or-fight’ response: heart rate, blood pressure, respiration and glucose synthesis are elevated, while body processes like digestion, immune system, mood and emotions, sexuality, and energy storage are decreased. These adaptive responses

aim to restore the homeostasis of the body's internal environment, and thus increasing chances for survival. If and how the stress system is triggered depends on the type and intensity of the stressor as sensed by the organism and interpreted in light of experience.[51] When activated the the hypothalamus sends impulses for activation of the stress regulating systems. In this thesis we will focus on two pathways of stress regulation: the HPA axis and the SNS.

Hypothalamic pituitary adrenal axis

At the onset of an acute stress response, the hypothalamus secretes corticotropin-releasing hormone (CRH). The portal vein transports CRH to (the anterior lobe of) the pituitary gland (a pea-shaped structure located below the hypothalamus) to induce the release of adrenocorticotrophic hormone (ACTH). Subsequently, ACTH acts on the adrenal cortices (small, conical organs on top of the kidneys) which produces the glucocorticoid hormone cortisol. Cortisol in turn acts on the hypothalamus and pituitary (to suppress CRH and ACTH production) in a negative feedback cycle.[52]

Figure 2 Schematic representation of the sympathetic nervous system (left arm) and the hypothalamic pituitary adrenal axis (right arm)



Sympathetic nervous system

A faster pathway to respond to acute stress is via the sympathetic nervous system (SNS). Upon activation, tyrosine is converted in the adrenal medulla into dopa which is subsequently converted into dopamine. Next, dopamine is transformed into norepinephrine, and then by N-methylation to epinephrine. The latter metabolic conversion is controlled by the enzyme phenylethanolamine N-methyltransferase (PNMT). For the transcription of PNMT high local concentrations of cortisol in the adrenal medulla are necessary. These concentrations of cortisol are supplied by the adrenal cortices (via stimulation of the HPA axis).[52] It is important to note that this contribution is just one example which illustrates that the SNS and the HPA axis are interrelated. Actually, both systems represent the effector limbs via which the brain influences all body organs during exposure to acute stress.[51]

Challenging the HPA axis and the SNS

In this thesis we will challenge the neuroendocrine system with the Trier Social Stress Test (TSST).[53] The TSST comprises a standardized protocol by which a subject is confronted with two stress tasks: a speech (mock job interview) and a mental arithmetical task in front of an audience/video camera. The specific characteristics of the TSST ('uncontrollability' and 'social-evaluative threat') induce stress responses which can be measured in neuroendocrine markers as cortisol (salivary-free cortisol and plasma cortisol (bound and free)), ACTH, norepinephrine and epinephrine increases, as well as in physiological changes in heart rate and blood pressure.[53-55]

The sensitivity of peripheral blood cells to glucocorticoids and adrenergic stimulation

The ultimate effect of the target tissue of the neuroendrocrine system does not solely depend on absolute levels of glucocorticoids, norepinephrine or epinephrine, but also on the sensitivity of receptors for these mediators on the target tissues. Given the fact that peripheral blood cells express receptors for all hormones and neurotransmitters allowing the cells to respond to signals given by the HPA axis and the SNS, we will also test the sensitivity of the peripheral blood cells to regulation by the glucocorticoid receptor agonist dexamethasone and the β2-adrenergic receptor agonist terubutaline. It is well established that glucocorticoids will inhibit the proliferative response of T cells.[56;57] Therefore, we will determine the effect of different concentrations dexamethasone on T cell proliferation and test whether chronic fatigue in sarcoidosis patients in clinical remission is associated with a change in the reactivity of the glucocorticoid receptor in T cells to respond to dexamethasone. Furthermore, terbutaline (which is a synthetic antagonist for the β2-adrenergic receptor and more stable than epinephrine), is known to regulate cytokine production by activated monocytes: in vitro it enhances the production of IL-10, and inhibits the production of TNF-α and other pro-inflammatory cytokines after stimulation with LPS.[56;59] We will therefore determine the LPS-induced production of IL-

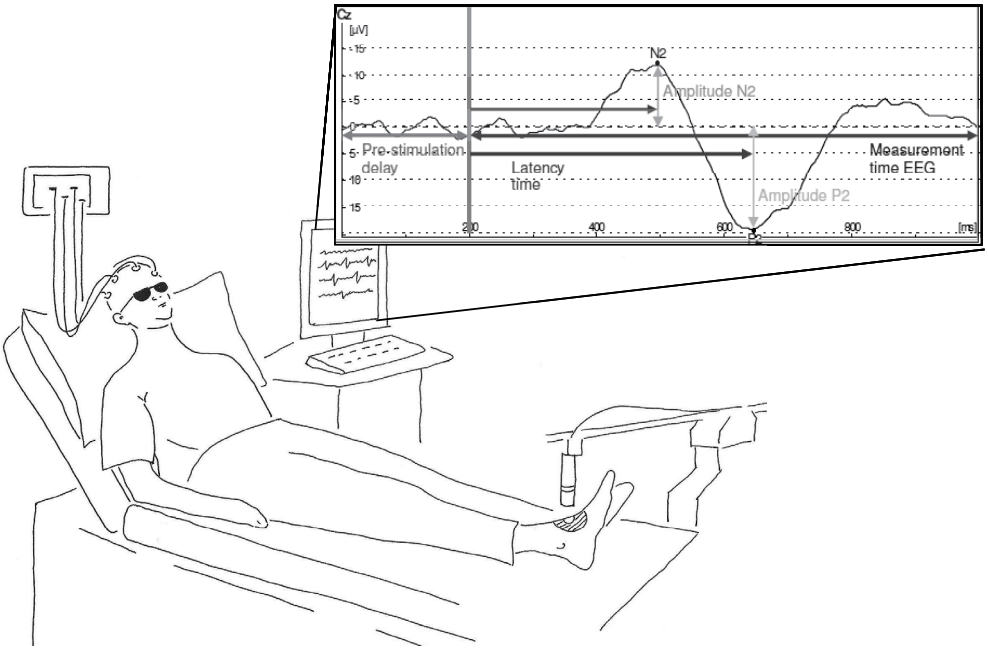
10 and TNF-α in the presence of increasing concentrations terbutaline, and test whether this production differs between the fatigued and the non-fatigued sarcoidosis patients in clinical remission.

Nociception by peripheral small fibers

From clinical observations it has become clear that chronically fatigued sarcoidosis patients after clinical remission of the disease also suffer from chronic pain, especially in hands and feet. Studies in active sarcoidosis patients complaining of pain, have suggested that sarcoidosis may induce a loss of small fibers.[60-62] Therefore, the next focus in the search for factors that might be associated with chronic post-inflammatory fatigue in sarcoidosis is on differences in the nociceptive (pain) processing by peripheral nerve fibers between fatigued and non-fatigued patients.

Pain is one of the most important sensations in daily life, and is defined as: "*an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage*".[63] The system that transports signals of damage and pain from the tissues via the fibers of the nervous system to the brain is referred to as the nociceptive system. Receptors of the nociceptive system (nociceptors) are densely localized in the surface of the skin, and use two different pathways to transport these signals: Aδ fibers and C fibers. Each type of fiber has its own characteristics and function. The Aδ fibers have a small diameter, are thinly myelinated, and conduct sensations of pain induced by heat (> 46 °C) or intense pressure on the skin with a rate of about 20 m per second towards the central nervous system. The C fibers are thicker and unmyelinated, which makes them slower in conducting pain sensations (around 1.5 m per second) but they are activated at a lower temperature (40 °C). Aδ and C fibers distinct different pain sensations: Aδ fibers make a sharp 'first', well localized pain sensible, while C fibers are responsible for prolonged aching, burning 'second' and more diffuse pain.[64;65] A lesion or dysfunction in the nociceptive system induces a particular type of pain, which is referred to as neuropathic pain.[66]

Figure 3 Experimental setup of Laser Evoked Potentials, with inset of a EEG recording of a typical LEP signal



In this thesis we will test Aδ nociceptive processing with laser evoked potentials (LEP): a 980-nanometer diode laser releases controllable heat stimuli in various intensities on the dorsum of the foot, while the evoked potentials are recorded by using standard electroencephalogram techniques. Consequently, the nociceptive function of neural pathways can be quantified by calculating the area under the curve (AUC), amplitudes and latencies of the laser evoked potentials. LEP is a method that is regularly applied for diagnostic and research purposes in the St. Antonius Hospital Nieuwegein.[67]

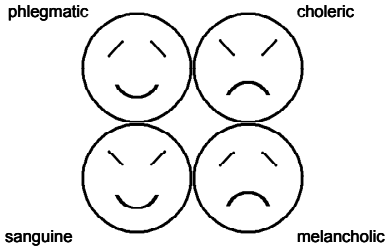
Personality and psychological symptoms

In our search for psycho-biological factors that might be associated with chronic fatigue in sarcoidosis in clinical remission, we lastly focus on personality traits and psychological symptoms.

Definition

The word 'personality' is derived from the Latin word 'persona' which means mask. In the theatre of the ancient world these masks were used to typify different characters. From this period of time may also date the first theoretic model of personality: the doctrine of the four humors, attributed to Hippocrates and Galen.[68] The balance of these four humors (sanguis/blood, phlegma/mucus, choler/bile, and melan choler/black bile) was believed to determine one's temperament: respectively sanguine, phlegmatic, choleric, or melancholic. In turn, temperament determined vulnerability to illness, and a humoral imbalance could lead to (physical or mental) illness. Balance (and thus health) was restored by techniques as bleeding and purging.

Figure 4 Emoticons to illustrate the four different temperaments



Cloninger's psychobiological model

Today, various personality models exist of which the Five Factor model, Eysenck's three-factor model of personality, and Cloninger's psychobiological model are the best known.

In this thesis we will use Cloninger's psychobiological model to identify personality traits. According to Cloninger[69], personality contains two components: temperament and character. Temperament is regarded as the biological aspect of personality as it is genetically inherited and emerges early in life. Processes such as memory, habit formation, emotional response and information processing are all influenced by temperament. Character development on the other hand is a continuous process that is influenced by life experience. Cloninger's model consists of four main temperaments: Novelty Seeking, Harm Avoidance, Reward Dependence and Persistence, and three character dimensions: Self Directedness, Cooperativeness and Self-transcendence. These dimensions of personality can be assessed with the Temperament and Character Inventory. In this study, we will use the short form.

Psychological distress

Some personality characters seem to be strongly correlated with psychological distress.[70;71] For example, the interrelation of depression and anxiety disorders with Harm Avoidance has been confirmed in several studies.[72-79] Therefore, in addition to personality traits, we will also assess symptoms of psychological distress such as depression, obsessive-compulsive behavior and anxiety.

Outline of the thesis

The first aim of this thesis is to determine the severity of chronic fatigue in sarcoidosis patients when the disease is in clinical remission, and to describe the characteristics of this post-inflammatory fatigue (**chapter 2**).

Next, we will focus on psycho-biological factors that might be associated with this chronic post-inflammatory fatigue. First, in **chapter 3**, we will investigate cytokine/chemokine profiles by comparing in vitro cytokine/chemokine production of peripheral blood leukocytes and plasma cytokines/chemokines between chronically fatigued and non-fatigued patients with sarcoidosis in clinical remission.

After that, in **chapter 4**, we will determine whether the neuroendocrine response to acute psychosocial stress differs between fatigued patients with sarcoidosis in clinical remission compared to a non-fatigued group of sarcoidosis patients in clinical remission. In addition, the sensitivity of the peripheral blood cells to the neuroendocrine signals will be tested.

Chapter 5 will test if Aδ nociceptive processing is reduced in chronically fatigued patients with sarcoidosis in clinical remission in comparison with non-fatigued patients and healthy controls.

Finally, we will explore in **chapter 6** if chronic post-inflammatory fatigue after clinical remission of sarcoidosis is associated with specific dimensions of personality, psychological symptoms and/or baseline levels of stress hormones.

The thesis will be concluded by a summary and discussion, including a short presentation of the results of a follow up study on the course of fatigue severity and an attempt to identify psycho-biological factors that may predict chronic post-inflammatory fatigue in sarcoidosis.

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2

Characterization of chronic fatigue in sarcoidosis in clinical remission

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Abstract

Background *Sarcoidosis patients frequently complain of fatigue, even when sarcoidosis has come into clinical remission. Primary aim of this study was to assess the severity of fatigue in sarcoidosis patients in clinical remission and to characterize it according the international criteria for Chronic Fatigue Syndrome (CFS). Furthermore, we evaluated whether fatigue is associated with depression and anxiety, health status, and patient-reported sleep quality, and recorded physical activity levels and muscle strength as objective assessments of fatigue.*

Methods *Data on 75 patients with sarcoidosis in clinical remission were obtained by questionnaires (Checklist Individual Strength, Symptom Checklist-90, Beck Depression Inventory-primary care, Medical Outcome Score-short form), standardized interview (CFS-criteria), sleep diary, accelerometer and muscle strength tests.*

Results *Fatigue severity mean score in sarcoidosis patients in clinical remission was high (CIS-fatigue-severity: 30.5 ± 15.5), and criteria for CFS were met in 47% of fatigued participants. Median time since diagnosis was 9 years. Fatigue was associated with depression ($p = 0.01$), anxiety ($p = 0.013$) and reduced health status ($p < 0.001$). Scores on sleep quality were normal. Physical activity levels were reduced in fatigued participants. Also muscle strength – particularly handgrip ($p = 0.006$) and quadriceps strength ($p < 0.001$) – was significantly associated with fatigue.*

Conclusions *Fatigue in sarcoidosis patients in clinical remission is a frequent symptom and can be characterized as a severe and long-lasting problem, symptomatically similar to CFS. Psychological distress and reduced health status are associated with fatigue. Interestingly, we observed significantly reduced physical activity and muscle weakness in fatigued patients.*

Introduction

Fatigue appears to be one of the most commonly reported complaints of patients suffering from sarcoidosis.[1-6] Not only at the onset and during the active phase of this multi-systemic granulomatous disorder, but also when clinically no disease-activity can be detected anymore, fatigue may persist.
This persistent fatigue had been labelled: post-sarcoidosis chronic fatigue syndrome, and the symptoms were described as: "…*profound symptoms of myalgia, fatigue, sleep reversal and low-spiritedness. The symptoms are out of proportion to the lack of physical signs and the absence of objective evidence of sarcoidosis*…"[7] Since then, researchers have referred to the term post-sarcoidosis chronic fatigue syndrome. [8-10] However, to the best of our knowledge no study is known that has investigated the exact features of fatigue in case of sarcoidosis has clinically come in remission.
Still, doctors are recurrently confronted with sarcoidosis patients with quiescent disease who suffer from a constellation of symptoms. Persistent and disabling fatigue is their most prominent complaint, often leading to reduced quality of life and labor disputes for incapacity to work.

From literature we know that post-infectious or post-inflammatory fatigue is a common feature. For example, more than 40% of the inflammatory bowel disease (IBD) patients in remission suffer from fatigue.[11] Similar reports are found in rheumatoid arthritis[12], infectious mononucleosis[13], cancer survivors[14;15] and Q-fever.[16] The pattern of symptoms found in all these conditions matches surprisingly well, and seems to be similar to that seen in Chronic Fatigue Symdrome (CFS)[17], although in these cases a known precipitating disease process is present.

The aim of this study was to determine the severity of fatigue after sarcoidosis, and to characterize it according to the international criteria for CFS.[17]
Moreover, we assess whether fatigue is associated with depression and anxiety symptoms, patient-reported sleep quality, and health status. Finally, this study records physical activity levels and muscle strength as objective assessments.

Methods and materials

Study Population

One hundred ninety-three sarcoidosis patients were invited to participate in the study either during their consult at the outpatient clinic of the St. Antonius Hospital Nieuwegein, or by letter from their former treating pulmonary physician. They were selected from a database of 800 sarcoidosis patients, irrespective of the presence of complaints of fatigue. Selection criteria were: sarcoidosis had been diagnosed according to the latest ATS/ERS/WASOG statement on sarcoidosis[18]; probability to meet the in- and exclusion criteria (see Table 1) on the basis of available medical records. Out of 193 patients, 115 responded to the invitation, and 88 of them were eligible for screening. After signing the informed consent, they were tested for in- and exclusion criteria. The inclusion criteria focus on the clinical remission of sarcoidosis, whereas the exclusion criteria rule out other causes and disabilities which might also explain the presence of fatigue. All criteria are listed in Table 1.

After screening, 75 (out of 88) participants were included. Thirteen participants were excluded because of: signs of active disease (increased serum sIL-2R or ACE or calcium or CRP) (8); Löfgren's syndrome as disease onset (2); thyroid disorder (1); diabetes (1) and one patient judged participation as too demanding.

The Medical Research Ethics Committee of the St. Antonius Hospital Nieuwegein, the Netherlands approved the study protocol, MEC ID: R-06.38A, GOV ID: NL14786.100.06.

Table 1 In- and exclusion criteria

Inclusion criteria:	
Previously involved organs	no clinical signs of disease activity
Serum parameters (soluble interleukin-2 receptor (sIL-2R), angiotensin converting enzyme (ACE), calcium^{2+}, C-reactive protein (CRP))	normal
Chest röntgenogram	normal or ≥2 years stable
Lung function	normal or ≥2 years stable,i.e.:
VC or FEV_1	<10% change
DLco	<15% change
Exclusion criteria:	
Löfgren's syndrome	at onset of sarcoidosis
Use of corticosteroids or other immunosuppressive drugs	over the last 6 months
Antidepressive medication	over the last 3 months
Psychiatric diseases (patient-reported)	
Sleep apnoea, narcolepsy or restless legs syndrome (by anamneses)	
Hemoglobine, leucocyte count, electrolytes, creatinine, serum calcium, liver enzymes, glucose, protein electrophoresis, ferritine, thyroid stimulating hormone (TSH), routine urine analysis[38]	abnormal
Body Mass Index (BMI) (kg/m^2)	<17 or ≥35
Abuse of alcohol, drugs or other substance (by anamneses)	
Age	>65 years

VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity of the lung for carbon monoxide

Measurements

Fatigue, health status, depression, and anxiety were measured with questionnaires (respectively: Checklist Individual Strength (CIS)[19]; Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36)[20;21]; Beck Depression Inventory for primary care (BDI-pc)[22]; Symptom Checklist-90 (SCL-90)[23;24]). The CIS is a questionnaire that completely focuses on fatigue and possesses excellent psychometric properties. The CIS is widely used to assess fatigue in other diseases as well.[19] Therefore the use of this questionnaire enables comparison of fatigue scores associated with different conditions.

Criteria for CFS were evaluated according to the international guidelines stated by the Centers for Disease Control and Prevention (CDC, CDC-criteria for CFS)[17] by means of a standardized interview. Criteria are summarized in Table 2.

Data on sleep quality were obtained by sleep diary (7 days).

Levels of physical activity were registered by an accelerometer (Actilog V3.0).[25] Participants were instructed to wear this motion device on the ankle during 14 consecutive days and nights, except whilst bathing, swimming and activities in rainy weather conditions. Registrations

shorter than 7 days were omitted from further analysis. A cut-off of 91 accelerations per day was considered as average general physical activity level.[25] Values for activity levels on weekdays and levels during the weekend were analyzed separately. Respiratory muscles strength tests were tested by maximal inspiratory pressure (PImax) and maximal expiratory pressure (PEmax) performed with a Master Screen Body (Jaeger, Würtzburg, Germany), handgrip strength (dominant side) with Jamar (Sammons Preston, Bolingbrook, USA) and quadriceps peak torque (dominant side) with Cybex (Cybex International, New York, USA). All measurements on muscle strength were expressed as percentages of predicted values, normalized for age, sex, height, and body weight.

Statistical analyses

After determining fatigue severity for the total study group, it was split in a fatigued and a non-fatigued group according to the standard cut-off for severe fatigue on the CIS-subscale *fatigue-severity* (≥35).[19] Prior to analysis all variables were tested for normality. Results are given in percentages, means ± standard deviations (SD), and medians and interquartile ranges [IQR]. Student's T-tests, Mann Whitney U-tests and binomial tests were performed to analyze differences between the two groups. The significance level for all analyses was set at $p < 0.05$. Data analysis was performed using Statistical Package for the Social Sciences version 16.0 (SPSS 16.0).

Table 2 Criteria for Chronic Fatigue Syndrome according to the international guidelines stated by the Centers for Disease Control and Prevention (CDC-criteria for CFS)[17]

Main criteria
(all must be met)
1. clinically evaluated but unexplained*
2. persisting or relapsing during 6 or more consecutive months
3. of new or definite onset (has not been lifelong)
4. not the result of ongoing exertion
5. not substantially alleviated by rest
6. resulting in substantial reduction in previous levels of occupational, educational, social, or personal activities
Fatigue-related symptoms
(four out of 8 must be met)
1. self-reported impairment in short-term memory or concentration, severe enough to cause substantial reduction in previous levels of occupational, educational, social, or personal activities
2. sore throat
3. tender cervical or axillary lymph nodes
4. muscle pain
5. multi-joint pain without swelling or redness
6. headaches of a new type, pattern, or severity
7. unrefreshing sleep
8. post-exertional malaise lasting more than 24 hours

* *In this study sarcoidosis was acknowledged as a precipitating trigger, even though disease activity at the time of study was clinically absent*

Results

Study population

Seventy-five patients with histopathologically proven sarcoidosis were included. In all patients no disease activity could be detected: serum parameters (ACE, sIL-2R, CRP, calcium) were normal; lung function was within norm scores. At the time of diagnosis 12 patients had extrapulmonary localisation of sarcoidosis, but physical examination at time of study revealed no clinical signs of disease activity in any organs. Two patients presented with slight bihilair lymphadenopathy (1 fatigued; 1 non-fatigued); röntgenogram revealed no abnormalities in all other patients. Median time since diagnosis was 9 years, with interquartile range of 5 to 17.

According to the cut-off score on the CIS-subscale *fatigue-severity* the total study group was divided in 2 groups. Thirty-eight participants (21 males; 17 females) were classified as non-fatigued; 37 as fatigued (12 males; 25 females). Characteristics of both groups are summarized in Table 3. No differences were found between both groups with respect to age, BMI, demographics, time since diagnosis, and history of corticosteroid use, except for gender: the fatigued group represented more females (25/37 versus 17/38 in the non-fatigued group). Therefore, all analyses were controlled for gender differences. In addition, we found a significant difference for one of the inflammation markers at disease onset: the non-fatigued group had had significantly higher serum ACE level than the fatigued group ($p = 0.006$), see Table 3.

Table 3 Characteristics of non-fatigued and fatigued group, in numbers, percentages, means ± SD,and medians [IQR]. The standard cut-off score of ≥35 for severe fatigue on the CIS subscale Fatigue severity was used to split the study population.

	Non-fatigued	**Fatigued**	p value
Number	38	37	
Fatigue severity	17.16 ± 7.02	44.69 ± 7.08	
Gender			**0.047**
male	21	12	
female	17	25	
Age	48.06 ± 7.4	46.25 ± 8.86	0.339
Body Mass Index (kg/m^2)	25.52 ± 2.91	26.28 ± 5.01	0.861
Marital status			0.534
single	4 (10.5%)	2 (5.4%)	
married	33 (86.8%)	31 (83.78%)	
divorced	1 (2.6%)	3 (8.1%)	
widowed and remarried	0 (0%)	1 (2.7%)	
Number of children			0.966
0	10 (26.3%)	11 (29.7%)	
1	4 (10.5%)	3 (8.1%)	
2 or more	24 (63.2%)	23 (62.2%)	
Time since diagnosis	10 [IQR: 5-18]	8 [IQR: 5.5-17]	0.689
Inflammationmarkers at onset*			
fever	3 (7 %)	3 (8%)	0.255
ERS (median, mm/h)	12 [IQR: 7-27]	9 [IQR: 5-28]	0.509
CRP (median, mg/l)	5 [IQR: 5-25]	5 [IQR: 5-18.5]	0.963
sACE (median, U/l)	66 [IQR: 48.5-100]	50 [IQR: 34-67.5]	**0.006**
Use of corticosteroids			
yes	13 (34.2%)	15 (40.5%)	0.637
period of corticosteroid use	21 months [IQR: 11-30]	11 months [IQR: 6-30]	0.761
Total number of affected organs	2 [IQR: 1-2.25]	1 [IQR: 1-2]	0.177

** Due to the fact that a number of patients were diagnosed with sarcoidosis in other hospitals, part of the data was missing. Number of patients in which data was available: fever: 33 non-fatigued and 29 fatigued; Eryhrocyte Sedimentation Rate (ERS): 31 non-fatigued and 30 fatigued; C-reactive protein (CRP): 23 non-fatigued and 20 fatigued; serum Angiotensin Converting Enzyme (sACE): 29 non-fatigued and 24 fatigued.*

Severity of fatigue

Overall, 76% of the total study population perceived severe complaints of fatigue during the onset and active phase of the sarcoidosis, and in 56% of these patients this fatigue persisted until current evaluation.

Evaluation of present feelings of fatigue in the total study group revealed a mean score of 30.5 ± 15.5 on the subscale *fatigue-severity* of the CIS. Performing a split based on the cut-off on this subscale showed a mean of 17.16 ± 7.02 for the non-fatigued group, and a mean of 44.69 ± 7.08 the fatigued group. No significant differences between mean scores of fatigued males and fatigued females were found (fatigued females: 45.76 ± 6.6 versus fatigued males: 43.08 ± 5.88, p = 0.20).

CDC-criteria for chronic fatigue syndrome

Seventeen out of 37 fatigued participants (47%) met the criteria completely. The presence of fatigue-related symptoms in non-fatigued and fatigued participants is shown in Table 4. All fatigue-related symptoms were significantly more frequent in the fatigued group. Interestingly, no significant differences were found between males and females, except for multi-joint pain, which was significantly more often reported by fatigued females (76% fatigued females versus 25% fatigued males, $p = 0.02$).

Table 4 Presence of fatigue-related symptoms of the CDC-criteria for chronic fatigue syndrome in non-fatigued and fatigued group, in percentages

	Non-fatigued	**Fatigued**	p value
Concentration/memory problems	13.2%	81.1%	< 0.001
Sore throat	18.4%	41.7%	< 0.05
Tender lymph nodes	10.5%	33.3%	< 0.05
Muscle pain	15.8%	72.2%	< 0.001
Multi-joint pain	10.5%	61.1%	< 0.001
Headaches	5.3%	55.6%	< 0.001
Unrefreshing sleep	18.4%	80.6%	< 0.001
Post-exertional malaise	5.3%	66.7%	< 0.001

Depression and anxiety symptoms

Mean score on the BDI-pc and on the SCL-90-subscale *Depression* were significantly higher in the fatigued group ($p < 0.01$; $p = 0.004$). Three participants (all fatigued) showed scores above the BDI-pc-cut-off of 4, indicating that they could be considered as suffering from clinical depression. However, supplementary analysis revealed that the high scores of these individual participants did not explain differences between both groups.

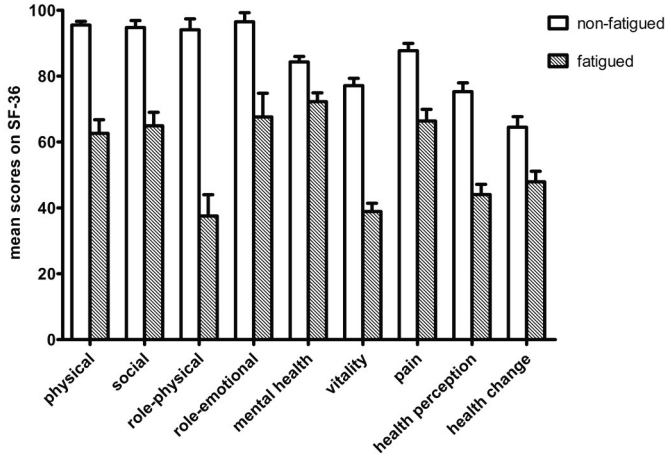
Mean scores on the SCL-90-subscale *Anxiety* showed significantly higher scores in fatigued participants in comparison with non-fatigued participants (non-fatigued: 11.89 ± 2.84; fatigued: 14.31 ± 4.81, $p = 0.013$). The differences between groups observed with BDI-pc and SCL-90 could not be explained by gender.

Health status

In all 9 dimensions of the generic health questionnaire SF-36 fatigued participants scored significantly lower (all $p < 0.001$, Figure 2). No differences between fatigued males and fatigued females were found, except for *Health status*: especially the fatigued males described their health as decreased compared to a year ago.

In addition, the dimension *Vitality* of the SF-36 (generally accepted as an accurate measurement of fatigue), correlated strongly with the CIS-subscale *fatigue-severity* (as used to identify the fatigued group in this study) ($r = -.684$, $p < 0.001$).

Figure 1 Mean scores on SF-36 in non-fatigued and fatigued group, all $p < 0.001$



Patient-reported sleep quality

Means of total self-reported night sleep time did not differ significantly between both groups (fatigued: 7:18h ± 51 min; non-fatigued: 6:46h ± 90 min, $p = 0.057$). Although there was a trend towards napping more minutes during daytime in fatigued participants (non-fatigued: 10 ± 20.49 minutes; fatigued: 22 ± 31.71 minutes), this difference did not reach statistical significance ($p = 0.052$). Moreover, the percentage of days that the fatigued group reported to awake unrefreshed was significantly higher (non-fatigued: 14.51 ± 17.66%; fatigued: 46.16 ± 28.74%; $p < 0.001$).

In all analyses gender did not explain the differences between groups.

Physical activity

Analysis included data of 32 non-fatigued and 29 fatigued participants. Fourteen registrations were omitted (7 shorter than 7 full days and nights, and 7 due to technical problems).

On weekdays the fatigued group achieved a mean value of 75.14 ± 24.09 accelerations per day versus 82.06 ± 27.69 in the non-fatigued group ($p = 0.30$), whereas on days during the weekend the mean values were respectively: 66.93 ± 29.43 (fatigued) and 79.81 ± 31.99 (non-fatigued) ($p = 0.10$).

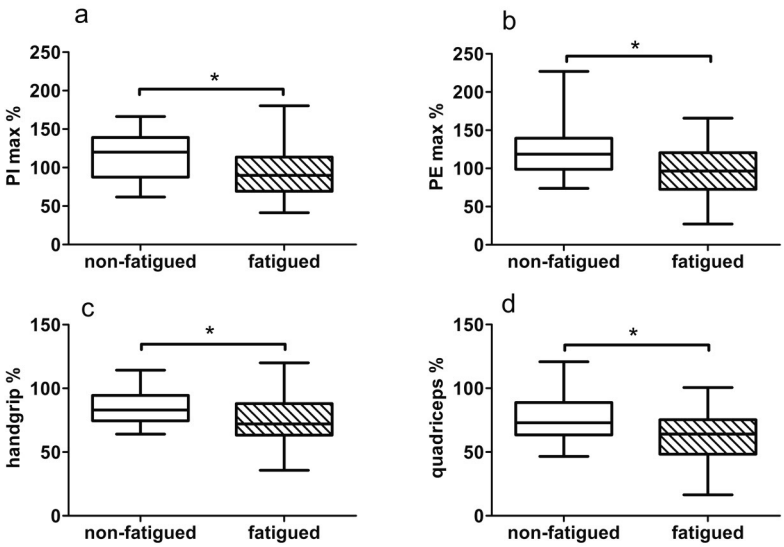
Compared with the norm score, we found activity levels in both groups below general norm score on weekdays and during the weekend. Mean values of the fatigued group, however, were significantly reduced compared to the norm score (weekdays: $p = 0.001$; weekend days: $p < 0.001$).

Muscle strength

Due to technical problems 3 participants could not complete the quadriceps muscle strength test (all non-fatigued).

Figure 2 shows the results of the four muscle strength tests: maximal inspiratory pressure (PImax), maximal expiratory pressure (PEmax), handgrip and quadriceps. On all tests the mean score of the fatigued group was significantly lower than the mean of the non-fatigued group.

Figure 2 Scores on (a) maximal inspiratory pressure (PImax), (b) maximal expiratory pressure (PEmax), (c) handgrip strength, and (d) quadriceps strength, expressed as a percentage of predicted values, in non-fatigued and fatigued participants, presented in boxplots with medians.



Differences between both groups were significant in all tests ($p < 0.006$).

Discussion

This study has shown that fatigue in sarcoidosis patients in clinical remission is severe. The CIS-*fatigue-severity*-scale revealed a mean score of 30.5 ± 15.5 for the total study population. According to the cut-off score of ≥35, 37 out of 75 patients could be classified as severely fatigued: their mean *fatigue-severity*-score was 44.69 ± 7.08. The CIS has also been used to measure fatigue in other (auto-)immune mediated diseases. Therefore the use of this questionnaire enabled us to compare fatigue severity with other diseases. Comparison of mean scores learns that fatigue severity in our study group is relatively high: multiple sclerosis:

40.2 ± 11.8[26], functional bowel disorders: 34.1 ± 8.5[19], cancer survivors: 21.1 ± 13.7.[27] Only in CFS patients show a higher CIS*fatigue-severity* mean score: 51.7 ± 4.6.[19;26]
So far, available studies on fatigue in sarcoidosis involve patients with clinically *active* disease. In a review of De Kleijn et al[6] the median incidence of fatigue in *active* sarcoidosis appeared to be 73% (IQR: 63-78%). Due to diversity of used questionnaires to assess fatigue, no clear picture of fatigue severity is available.
Although the interpretation and quantification of fatigue severity remains a subjective parameter which might even be disease and situation specific, we would like to propose that fatigue after sarcoidosis can be interpreted as a severe problem.

Secondly, evaluation of fatigue in sarcoidosis in clinical remission by applying the international CFS-criteria showed profound presence of fatigue-related symptoms such as concentration problems, pain, post-exertional malaise and unrefreshing sleep. The relevance of this last symptom was confirmed by data obtained by sleep diary. This pattern of symptoms matches remarkably well with those found in other post-inflammatory fatigue reports.[28]
In addition, we found 47% of our fatigued study group to fully meet the CFS-criteria, taken into account that in our study sarcoidosis was known as a precipitating trigger. Therefore, we propose that chronic fatigue is a long-term effect of sarcoidosis.

In this study we demonstrated that fatigue in sarcoidosis patients in clinical remission co-occurs with depressive and anxiety symptoms. Fatigued participants scored significantly higher than non-fatigued participants on all depression and anxiety scales. Neither gender differences, nor BMI and physical activity explained the enhanced levels of psychological distress.
Although depression and anxiety often accompany symptoms of prolonged fatigue[29;31], we do not know whether these symptoms found in our study are precipitating factors, since we have no information on psychological distress before or during the disease. Even so, it is difficult to judge whether the psychological factors are part of the symptom constellation of fatigue or whether they reflect residual disease (inflammatory activity).
Moreover, the observed reduction in health status is significantly associated with severe fatigue: the fatigued group experienced significant reduction in physical and social functioning, and indicated to experience more problems with respect to mental health, vitality and pain, due to their health. Even though we are unable to address this observation in terms of causes and consequences, we propose that psychological interventions in fatigued patients may profoundly improve their psychological and health status.

Objective measurements of fatigue comparison revealed reduced activity levels and muscle weakness in the fatigued group. The latter result is in contrast with findings in CFS-patients

who retain their capacity for short duration isometric tasks[32], but is in line with reports on muscle weakness in active sarcoidosis.[33] However, previous corticosteroid treatment (probably the cause in active disease) was not associated with muscle weakness in our study population. Taken these data together, we would like to hypothesize that sarcoidosis may induce muscle damage during the active phase of the disease, and that this myopathy will remain unrecovered, even though clinical signs of disease-activity are absent.

Some limitations of the study should be acknowledged. First of all, the criteria for clinical remission of sarcoidosis. As there is no international accepted golden standard, these criteria were based on clinical experience (JB and JG) and literature.[34;35] Therefore, we might unintentionally have included some participants with sarcoidosis at a subclinical level. Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography ((18)F-FDG PET)-scan, which is found a very sensitive technique to assess active sarcoidosis[36], may detect this low-grade disease activity. In our study 8 patients had been subjected to a (18)F-FDG PET-scan at the time of the study. Although none of these patients displayed any sign of disease, it would be interesting to test all fatigued participants in order to investigate if residual FDG uptake can be demonstrated in these patients.

In addition, one may speculate about the linkage with persistent fatigue and disease severity at onset. Although no differences were found in total number of affected organs, presence of fever, ESR and CRP between both groups at onset, the sACE concentrations at that time point were significantly higher in the non-fatigued group. This remarkable finding might suggest that a severe inflammation process at the start of the disease might contribute to recovery from fatigue. On the other hand, all other inflammation markers did not show significant differences, the median time since diagnosis had been already 9 years and serum ACE concentrations at the time of the study did not differ. However, the question remains open whether severe fatigue is in fact the most sensitive sign of a low-grade inflammation (that is up till now undetectable by current routine investigations) which remains present many years after clinical remission of the disease. This theory would well fit with the hypothesis that sickness behaviour, including fatigue and malaise, could well be induced by low-grade inflammation.[31]

Alternatively, we might speculate that the brain of the fatigued patient might have been sensitized by cytokines during the active phase of the disease process, leading to an alteration in the set point of regulatory systems involved in fatigue and depression.[37] The latter would imply that although the production of inflammatory mediators is the same as in healthy individuals, the sensitivity of receptors for immune mediators may be increased, leading to the observed phenomena of fatigue.

In summary, fatigue in sarcoidosis patients in clinical remission is a severe and long-lasting problem, which is accompanied by psychological distress and reduced health status. It co-occurs with concentration problems, pain, unrefreshing sleep and post-exertional malaise – a pattern similar to CFS. Interestingly, reduced physical activity and muscle weakness are observed in fatigued patients.

Although the mechanism of this post-inflammatory fatigue has still to be elucidated, the characteristics derived from this study will enable physicians to address fatigue and fatigue-related complaints in sarcoidosis patients in clinical remission more thoroughly, and to be more aware of the severity of the problem in individual cases.

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3

Reduced Th2 cytokine production by sarcoidosis patients in clinical remission with chronic fatigue

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Abstract

Rationale *When the inflammatory phase of sarcoidosis has resolved, complaints of chronic fatigue frequently persist. Low-grade residual inflammatory activity may play a role in maintaining chronic fatigue.*

Objectives *To compare in vitro cytokine/chemokine production and plasma cytokine/chemokine levels between chronically fatigued and non-fatigued patients with sarcoidosis in clinical remission.*

Methods *Patients with sarcoidosis in clinical remission were assigned to a non-fatigued group (n=38) or a fatigued group (n=34) based on the standardized cut-off of the fatigue questionnaire Checklist Individual Strength. Cytokines/chemokine in plasma and in supernatants of whole blood cultures stimulated with either a T cell mitogen or lipopolysaccharide were quantified by multiplex analysis.*

Associations of cytokine/chemokine profiles with chronic fatigue were analyzed by multivariate analysis of variance and principal component analysis followed by logistic regression.

Results *Principal component analysis of T cell mitogen-induced cytokine/chemokine production identified 3 components that explained 76% of the variance in the cytokine data. Logistic regression revealed that the 'Th2 cytokine'-component which mainly consists of interleukin (IL)-4, IL-5 and IL-10 was significantly and negatively associated with chronic fatigue.*

In addition, multivariate analysis revealed higher levels of LPS-induced IL-8 and lower levels of plasma monocyte chemoattractant protein (MCP)-1 in the fatigued group compared to the non-fatigued group.

Conclusions *In chronically fatigued sarcoidosis patients in clinical remission, we found a cytokine/chemokine profile which is suggestive for a less competent Th2 counterbalancing capacity, that may contribute to the persistence of chronic fatigue.*

Introduction

Sarcoidosis is a systemic disorder of unknown origin which is characterized by the development of granulomas in different organs. The disease commonly affects young and middle-aged adults. It usually resolves within 2 to 4 years.[1] In addition to the functional problems due to the impaired functioning of affected organs, sarcoidosis patients suffer from a range of non-specific symptoms of which fatigue is among the most reported ones.[2;3] Moreover, fatigue may even persist when clinically no signs of disease-activity are detectable any more. In a previous study[4] we showed that in our study cohort of sarcoidosis patients in clinical remission, chronic fatigue was present in 49% of the patients and had lasted up to 9 years after diagnosis. Moreover, fatigued patients with sarcoidosis in clinical remission also presented with fatigue-related symptoms such as concentration problems, pain, malaise after exertion and unrefreshing sleep. These fatigue-related symptoms are internationally accepted as criteria for the diagnosis of chronic fatigue syndrome (CFS).[5]

Since sarcoidosis is an immune mediated inflammatory disease, we wondered whether chronic fatigue may persist due to a residual low-grade inflammatory activity in these patients. During the active phase of sarcoidosis, activated Th1 cells and macrophages produce interleukin (IL)-2, interferon (IFN)-γ and tumor necrosis factor (TNF)-α.[6-9] Apart from the primary function of immune regulation, cytokines produced at inflammatory sites have often been associated with the existence of non-specific symptoms of disease also known as cytokine-induced sickness of which fatigue is a profound characteristic.[10-12] It has been shown in animal studies that peripheral administration of a cytokine inducer like lipopolysaccharide (LPS), or of a recombinant cytokine such as IL-1β or TNF-α induces symptoms of sickness behavior.[10] Conversely, administration of cytokine antagonists inhibits the physiologic and behavioral effects of LPS.[13;14] In humans sickness behavior has first been observed during administration of inflammatory cytokines like IFN-α as treatment in chronic hepatitis C[15;16] and in malignant melanoma.[17]

It has been suggested that a dysbalance in pro- and anti-inflammatory cytokines may contribute to the symptomatology of CFS.[18-20] However, mixed results with respect to cytokine patterns have been reported. After stimulation of peripheral blood cells in vitro, associations of chronic fatigue with Th1 cytokines[21], as well as with Th2 cytokine profiles[22-24] have been observed in CFS patients. Based on these findings in CFS and the similarities in symptomatology between patients complaining of chronic fatigue after clinical remission of sarcoidosis and CFS patients, we investigated the cytokine/chemokine profile in our cohort of sarcoidosis patients in clinical remission.

Methods

The Medical Research Ethics Committee of the St. Antonius Hospital Nieuwegein, the Netherlands approved the study protocol, MEC ID: R-06.38A, GOV ID: NL14786.100.06.

Study population

One hundred ninety-three sarcoidosis patients were selected from a database and invited to participate. Selection criteria were: diagnosis of sarcoidosis according to the latest ATS/ERS/WASOG statement on sarcoidosis[1]; probability to meet the in- and exclusion criteria (Table 1) on the basis of available medical records. Upon signing the informed consent, 88 responders were screened, and 75 of them were included.

Based on the standardized cut-off on the *fatigue-severity* subscale of the Checklist Individual Strength (CIS)[25], patients with a score ≥35 were allocated to the fatigued group; the others to the non-fatigued group.

Table 1 In- and exclusion criteria

Inclusion criteria:	
Previously involved organs	no clinical signs of active involvement
Serum parameters (sIL-2R, ACE, Ca^{2+}, CRP)	normal
Chest X-ray	normal or ≥ 2 years stable
Lung function tests:	normal or ≥ 2 years stable, i.e.:
VC or FEV_1	<10% change
DLco	<15% change
Exclusion criteria:	
Löfgren's syndrome	at onset of disease
Use of corticosteroids or other immunosuppressive drugs	over the last 6 months
Use of analgesic medication	over the last 3 months
Antidepressive medication	over the last 3 months
Psychiatric diseases (patient-reported)	
Sleep apnea, narcolepsy or restless legs syndrome	
Hb, Blood Cell Count, Na^{2+}, K^+, creatinine, GGT, AF, ASAT, ALAT, LDH, glucose, total protein, TSH, ferritine, routine urine analysis	abnormal
BMI (kg/m^2)	<17 or ≥35
Abuse of alcohol, drugs or other substance	
Age	>65 years

sIL-2R = soluble interleukin-2 receptor, ACE = angiotensin converting enzyme, Ca^{2+} = calcium, CRP = C-reactive protein, VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity of the lung for carbon monoxide, BMI = body mass index (kg/m^2)

Bloodsampling

All blood samples were collected between 10.30 and 11.00 AM, via an intravenous catheter after 30 minutes rest. Heparinized blood for in vitro stimulation was kept at room temperature; blood samples for determination of plasma cytokines were collected into ethylene diamine tetra acetic acid (EDTA) tubes on ice. Plasma was separated within 30 minutes and stored at -80 °C until analyzed.

T cell mitogen-induced cytokine/chemokine production

Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine was stimulated with anti-CD2/CD28 monoclonal antibodies (CLB, Amsterdam, Netherlands, final concentration anti-CD2.1/anti-CD2.2 0.33 μg/ml and anti-CD28 1.33 μlg/ml) for 72 hours at 37 °C/5% CO2 in 96-well round-bottomed plates. T cell mitogen-induced secretion of IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ, monocyte chemoattractant protein (MCP)-1 (also CCL2), interferon-gamma induced protein (IP)-10 and RANTES (CCL5) were measured in supernatants using multiplex cytokine assay as described before.[26]

Lipopolysaccharide (LPS)-induced cytokine/chemokine production

Whole blood, diluted 1:10 with RPMI-1640 (Gibco, Grand Island, NY), was stimulated with lipopolysaccharide (LPS, Escherichia Coli 0127:B8, Sigma, final concentration 1 ng/ml) for 24 hours at 37 °C/5% CO2 in 96-well flat-bottomed plates to activate monocyte cytokine production. Supernatants were analyzed by using multiplex assay for the presence of: IL-1α, IL-1β, IL-6, IL-8, IL-10 and TNF-α, as described.[26]

Plasma cytokines/chemokines

Plasma levels of IL-1α, IL-1β, interleukin 1 receptor antagonist (IL-1Ra), IL-5, IL-6, IL-8, IL-10, TNF-α, MCP-1, RANTES, macrophage inflammatory protein-1α (MIP-1α), and macrophage inflammatory protein-1β (MIP-1β) were assessed in plasma by multiplex assay (R&D Systems Europe Ltd., Abingdon, UK), according to the manufacturer's instructions.

Statistical analysis

As cytokines may emerge in functional clusters, we bundled the effect of this intercorrelation by principal component analysis (with varimax rotation) on the T cell mitogen-stimulated data set. Only components with initial eigenvalues >1 were selected. Loadings >0.6 were used to identify the variables comprising a component. Logistic regression (enter method) was used to explore the association of the components with chronic fatigue.

The number of cytokines analyzed after LPS-stimulation and detectable in plasma was too small to conduct a proper principal component analysis and multivariate analysis was used

instead. All cytokines were log transformed to approximate the normality assumptions. Data analysis was performed using SPSS 16.0.

Results

Study population

Seventy-two out of 75 patients were included in the present study, because blood sampling failed in 3 subjects (all fatigued). In line with the inclusion criteria, no disease activity was detected in all patients: serum parameters (ACE, IL2-R, CRP, calcium) and lung function were normal; physical examination revealed no clinical signs of disease activity in any organ anymore. One patient presented with slight but stable bihilar lymphadenopathy; chest X-ray revealed no abnormalities in all other patients. Median time since diagnosis was 10 years, with an interquartile range of 5 to 18 years.

According to the standardized cut-off of the *fatigue-severity* subscale of the CIS [25], patients who scored ≥35 were assigned to the fatigued group (n=34); all other patients were assigned to the non-fatigued group (n=38). No significant differences were found between both groups with respect to gender, age, BMI, demographics, time since diagnosis, history of corticosteroid use, and serum parameters at inclusion (Table 2). Although the group difference in gender distribution did not reach statistical significance ($p = 0.061$), all analyses were corrected for gender.

Table 2 Characteristics of the study population

	Non-fatigued group	Fatigued group	p value
Number	38	34	
Fatigue severity (CIS-subscale score)	15.16 ± 7.02	44.53 ± 6.41	
min - max	8 - 32	35 - 56	
Gender			0.061
male	21	11	
female	17	23	
Age	48 ± 7	46 ± 8	0.267
min - max	32 - 60	30 - 65	
BMI	25 ± 3	25 ± 4	0.844
Marital status			0.464
single	4 (10.5%)	2 (5.9%)	
married/cohabitant	33(86.8%)	29 (85.3%)	
divorced	1 (2.6%)	3 (8.8%)	
Highest level of education			0.356
primary	0 (0%)	2 (5.9%)	
secondary	11 (28.9%)	6 (17.6%)	
upper secondary	14 (36.8%)	14 (41.2%)	
tertiary/higher	13 (34.2%)	12 (35.3%)	
Smoking			0.659
yes	5 (13.1%)	6 (17.6%)	
former	14 (36.8%)	9 (26.5%)	
never	19 (50%)	19 (55.9%)	
Time since diagnosis (median in years and [IQR])	10.0 [5-18]	10.0 [6-17]	0.932
History of corticosteroids			
yes	13 (34.2%)	13 (38.23%)	0.808
period of use (median in months and [IQR])	21 [11-30]	11 [6-30]	0.806
Localistation at diagnosis			0.53
pulmonary	33 (86.84%)	27 (79.41%)	
extrapulmonary	5 (13.15%)	7 (20.58%)	

Data represent numbers and percentages, means ± SD, and medians and and [interquartile ranges (IQR)]

T cell mitogen-induced cytokine/chemokine production

Table 3 summarizes the medians and interquartile ranges (IQR) of 10 cytokine/chemokine concentrations in the supernatants of whole blood cultures stimulated in vitro with the T cell mitogen anti-CD2/CD28 of the non-fatigued and the fatigued group.

Table 3 T cell mitogen-induced cytokine/chemokine production in the non-fatigued group and the fatigued group

	Non-fatigued group		Fatigued group	
	median	IQR	median	IQR
IL-2	379	130-710	376	130-1256
IL-4	60	44-128	42	19-67
IL-5	618	240-1106	342	181-805
IL-6	294	72-430	436	124-1027
IL-10	380	165-608	223	110-407
TNF-α	565	272-937	481	175-1093
IFN-γ	1234	325-2998	1044	88-5249
MCP-1	5295	3648-7286	5233	4001-9928
IP-10	3019	1928-3974	2656	2026-5628
RANTES	2111	1204-3403	2552	1382-3639

Data represent median and [interquartile range (IQR)], in pg/ml

Principal component analysis of these data identified three components that together explained 76.3% of the total variance in the cytokine data (Table 4). Notably, these three components represent functional clusters of cytokines/chemokines. The first component was labeled 'Th1 cytokines' as it principally consisted of IFN-γ, TNF-α, RANTES, IL-2, and IL-6. The second component predominantly yielded IL-4, IL-5 and IL-10 and was therefore referred to as 'Th2 cytokines'. MCP-1 and IP-10 characterized the third component: 'Chemokines'.

Table 4 Principal component analysis of T cell cytokines/chemokines

	'Th1 cytokines'	**'Th2 cytokines'**	**'Chemokines'**
IFN-γ	**0.889**	0.298	-0.020
TNF-α	**0.840**	0.375	0.051
RANTES	**0.785**	0.068	0.057
IL-2	**0.728**	0.337	0.097
IL-6	**0.682**	-0.221	0.551
IL-4	0.126	**0.926**	-0.095
IL-5	0.169	**0.839**	0.122
IL-10	0.324	**0.768**	-0.46
MCP-1	-0.079	-0.180	**0.905**
IP-10	0.218	0.323	**0.685**
Eigenvalue	4.424	1.990	1.214
% of variance	44.235	19.889	12.136

** Rotated (Varimax method) Component Loadings >0.6 in bold*

Logistic regression analysis was used to determine whether cytokine/chemokine characteristics could predict whether the individual belonged to the fatigued or the non-fatigued group. The 'Th2 cytokines' were significantly and inversely associated with chronic fatigue (B = - 0.919, p = 0 .007). Both the 'Th1 cytokines' and the 'Chemokines' did not emerge as significant predictors of chronic fatigue (p = 0.078 and p = 0.215, Table 5).

Table 5 Logistic regression analysis of three components on chronic fatigue

			95% CI for Odds Ratio		
	B (SE)	**Odds Ratio**	**Lower**	**Upper**	p value
'Th1 cytokines'	0.576 (0.327)	1.778	0.938	3.373	0.078
'Th2 cytokines'	- 0.919 (0.339)	0.399	0.205	0.776	**0.007**
'Chemokines'	0.374 (0.301)	1.453	0.805	2.624	0.215

LPS-induced cytokine/chemokine production

Multivariate analysis of cytokines/chemokines (IL-1α, IL-1β, IL-6, IL-8, IL-10 and TNF-α) produced after in vitro stimulation of whole blood cultures with LPS showed that only IL-8 differed significantly between non-fatigued and fatigued individuals. The fatigued group produced significantly more IL-8 as compared to the non-fatigued group (p = 0.047, Table 6).

Table 6 LPS-induced cytokine/chemokine production and plasma cytokines/chemokines

	Non-fatigued group	**Fatigued group**	p value
LPS-induced			
IL-1α	36 [21-59]	38 [26-55]	0.623
IL-1β	169 [103-251]	149 [91-247]	0.680
IL-6	1780 [1056-2601]	1561 [1082-2199]	0.666
IL-8	1735 [1524-1842]	1822 [1647-1949]	**0.047**
IL-10	13 [7-19]	14 [9-25]	0.238
TNF-α	263 [183-432]	240 [183-353]	0.872
Plasma			
IL-1β	0.96 [0.55-0.96]	0.55 [0.55-0.96]	0.335
IL-1Ra	402 [328-596]	387 [279-538]	0.264
IL-8	4 [2-5]	3 [1-4]	0.094
MCP-1	114 [100-131]	91 [81-106]	**0.009**
MIP-1β	26 [21-36]	28 [15-33]	0.052
TNF-α	3 [2-4]	3 [2-4]	0.566

Data represent median and [interquartile range(IQR)] in pg/ml and were analyzed by MANOVA, and corrected for gender. Statistical significant differences in bold.

Cytokines/chemokines in plasma

In plasma the level of 5 out of 11 cytokines/chemokines were under the detection limit in the majority of samples. IL-1α was not detectable in 95.8% of all patients, IL-5 in 90.3%, IL-6 in 75%, IL-10 in 91.7% and MIP-1α in 70.8%. The percentage of individuals with plasma levels below detection limits for these cytokines/chemokines did not differ between the non-fatigued and the fatigued group. Data for these 5 cytokines/chemokines were excluded from further analysis. Multivariate analysis of the other 6 cytokines/chemokines (IL-1β, IL-1Ra, IL-8, MCP-1, MIP-1β and TNF-α) showed that plasma of individuals in the fatigued group contained significantly lower levels of MCP-1 compared to the non-fatigued group ($p = 0.009$, Table 6).

Discussion

This is the first report describing cytokine/chemokine profiles in patients with chronic fatigue after clinical remission of the inflammatory disease sarcoidosis. We investigated cytokine/cytokine profiles in a group of 72 patients in clinical remission, of whom 49% reported severe fatigue even 10 years after diagnosis. Our data show that the capacity to produce Th2 cytokines (IL-4, IL-5 and IL-10) in vitro is decreased in chronically fatigued patients with sarcoidosis in clinical remission compared to non-fatigued patients in clinical remission. Furthermore, we observed higher levels of LPS-induced IL-8 production as a parameter of innate immune function, as well as lower levels of the chemokine MCP-1 in plasma of the chronically fatigued group in comparison with the non-fatigued patients in clinical remission.

We performed principal component analysis before analyzing the data on production of cytokines/chemokines in response to T cell stimulation in vitro. This principal component analysis led to the identification of three components that together explained 76.3% of the total variance in the cytokine data. The latter result is in line with data from previous studies[27;28] and supports the notion that principal component analysis is a good way to describe multiplex cytokine profiles.

Active ongoing inflammation is known to be associated with fatigue. Pro-inflammatory cytokines will act on the brain during inflammation to cause a behavioral response called sickness behavior. Cytokine-induced sickness behavior has been well defined and studied in animal models.[29;30] In humans, fatigue is an important component of sickness behavior.[31] In principle, sickness behavior represents part of the normal defense against infections or tissue damage. However, it has been postulated that in vulnerable individuals, this adaptive sickness response may evolve into maladaptive chronic behavioral abnormalities, including chronic fatigue and depressive behavior.[32] Studies on administration of cytokines as therapy for different diseases confirm this view. For example, IFN-α therapy in hepatitis C patients induces major depressive

disorder (MDD) in 30-40% of the patients.[15] Pre-treatment circulating levels of the cytokine IL-6 have been shown to be highly predictive for the incidence of MDD in these IFN-α-treated hepatitis C patients.[33] Conversely, treatment with TNF-α antagonists induced a rapid decrease in fatigue symptoms in patients with the chronic inflammatory disease rheumatoid arthritis [34;35] as well as in inflammatory bowel disease.[36]

In our study, the most intriguing finding is that chronic fatigue in patients with sarcoidosis in clinical remission was associated with reduced production capacity of Th2 type cytokines. The first phase of sarcoidosis is dominated by a profound Th1 cytokine profile. Subsequently, the phase after granuloma formation is characterized by a skewing to a dominance of Th2 cytokines in the alveoli.[37;38] This temporary Th2 cytokine dominance is thought to be beneficial because it may limit the ongoing pro-inflammatory response. Notably, we have shown before that the fatigued group had a lower serum ACE level at the time of diagnosis of the disease than the non-fatigued group.[4] These findings indicate that during active disease, the fatigued group had a reduced pro-inflammatory profile as compared to the non-fatigued group.
It is possible that the lower capacity of fatigued patients to produce Th2 as we observed in our study, is due to the specific genetic background, although it is highly unlikely that almost 50% of the population would carry the polymorphism(s) associated with low Th2 cytokine production.

Our finding that plasma levels of MCP-1 in the fatigued group are decreased in comparison to the non-fatigued group of patients with sarcoidosis in clinical remission is novel and intriguing. MCP-1 is known to actively regulate Th2 cytokine expression in a way that it upregulates Th2 cytokine production.[39] The latter data may imply that the low Th2 cytokine production in fatigued patients is causally related to decreased MCP-1 production. Moreover, we cannot exclude that there is still residual subclinical ongoing inflammatory activity in the lungs of the fatigued group of patients. We do not know at present whether the decreased MCP-1 levels or the increased production of IL-8 are a reflection of an ongoing low-grade immune activation by the disease process. In this respect it is of interest that we observed an increase IL-8 response after stimulation with LPS, which mainly activates monocytes/macrophages in this system [40] reflecting a pro-inflammatory monocyte/macrophage phenotype in the fatigued group in the peripheral circulation.

In the context of our current findings one might wonder whether chronically fatigued patients are really in complete remission and whether severe fatigue in sarcoidosis patients after ‘clinical remission’ may in fact reflect the most sensitive parameter of residual disease activity. In a preliminary follow up study of our patient group, one year after the blood sampling, we observed that chronic fatigue was stable and persistent over time, and that the reduced capacity

to produce Th2 cytokines is a strong predictor of chronic fatigue at follow up (Korenromp et al., in preparation). The persistence of sickness behavior or severe post-inflammatory fatigue may be a crucial factor for development of clinical depression as has been shown in animal models [41] and in humans.[42] Therefore we are inclined to suggest that the symptoms of chronic fatigue should be taken seriously and should be treated with medication aimed at restoring Th1/Th2 balance, preferably in combination with behavioral intervention. Such interventions may help to improve the quality of life of the fatigued sarcoidosis patient 'in clinical remission'. In conclusion, this is the first study that shows that chronic fatigue in sarcoidosis in clinical remission is associated with a change in cytokine/chemokine profiles.

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4

Reactivity of the sympathetic nervous system and HPA axis to acute stress of patients with sarcoidosis in clinical remission

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Abstract

The etiology of chronic fatigue after clinical remission of the inflammatory disease sarcoidosis is still unknown. It is now well established that there is a bi-directional communication between the immune system and the hypothalamus pituitary adrenal axis or the sympathetic nervous system. Dysregulation of one of the systems may influence immune functioning and the disease outcome of sarcoidosis.

In 71 sarcoidosis patients in clinical remission we determined the neuroendocrine response to acute stress (Trier Social Stress Test (TSST)). Plasma concentrations of adrenocorticotropic hormone (ACTH), cortisol, norepinephrine, epinephrine, and blood pressure and heart rate over time were compared between fatigued (n=34) and non-fatigued patients (n=37). Additionally, the sensitivity of the peripheral blood cells to the glucocorticoid dexamethasone and the β2-adrenergic agonist terbutaline were determined.

Fatigued females showed no significant change in epinephrine levels in response to the TSST, compared to non-fatigued females. In fatigued males, TSST elicited an epinephrine increase only during the anticipatory phase of the test. Fatigued females showed a lower blood pressure during the whole test compared to non-fatigued females. In both genders, we did not observe differences between the fatigued and the non-fatigued group in stress-induced changes in norepinephrine, heart rate, ACTH and cortisol.
Furthermore, the sensitivity of peripheral blood cells of fatigued female patients to regulation by terbutaline was decreased with respect to production of IL-10 when compared to non-fatigued female patients.

In summary, chronic fatigue after clinical remission of sarcoidosis is associated with a dysregulation of a stress-induced epinephrine secretion, while the responsiveness of norepinephrine and the HPA axis seems to be intact.

Introduction

Sarcoidosis is a systemic inflammatory disorder characterized by granuloma formation in different organs. Lungs, lymphatic system, skin and eyes are most frequently affected. Liver, spleen, lymph nodes, salivary glands, heart, nervous system, muscles, bones, and other organs may also be involved. Sarcoidosis is characterized by an activation of the T helper 1 cells (Th1) that induces the secretion of predominantly interleukin (IL)-2 and interferon-gamma (IFN-γ), followed by macrophage tumor necrosis factor-alpha (TNF-α) production. Sarcoidosis commonly affects young and middle-aged adults, and usually resolves within 2 to 4 years.[1]

Besides functional problems due to the affected organs, patients suffer from a range of non-specific conditions such as fatigue, fever, arthralgia, and depressed mood.[2] Remarkably, complaints of fatigue often persist in a substantial subgroup of patients, even when clinically no disease activity can be found anymore. In a previous study[3] we showed that this post-inflammatory fatigue was present in 49% of our study group which consisted of sarcoidosis patients in clinical remission, with a median of 9 years after diagnosis. In 47% of the fatigued cases their symptom profile met the internationally accepted criteria for Chronic Fatigue Syndrome (CFS)[4], taking into account that sarcoidosis was acknowledged as precipitating trigger for their fatigue. Furthermore, we found that chronic fatigue was significantly associated with psychological distress, reduced health status, chronic pain, reduced physical activity and muscle weakness. In summary, we characterized chronic fatigue in patients with sarcoidosis in clinical remission as a severe and disabling problem that is symptomatically similar to CFS. However, the mechanism underlying this post-inflammatory chronic fatigue still remains unclear.

Since sarcoidosis is an inflammatory disease and bi-directional communication between the immune system and the neuroendocrine system is well acknowledged[5;6], we raised the question whether the neuroendocrine system might be involved in the maintenance of the post-inflammatory fatigue. The immune system and the neuroendocrine system communicate via numerous routes of which the hypothalamic pituitary adrenal (HPA) axis and the sympathetic nervous system (SNS) are important pathways. In the present study we tested the responsiveness of the HPA axis and the SNS by subjecting participants to acute stress by means of the Trier Social Stress Test (TSST).[7]

We investigated whether the neuroendocrine response to this stressor differed between sarcoidosis patients in clinical remission who reported chronic fatigue and a group of sarcoidosis patients in clinical remission who belonged to the non-fatigued group. However, the functioning of the neuroendocrine system is not only determined by the level of secretion of the hormones or neurotransmitters, but also by the sensitivity of the receptor for a particular neuroendocrine mediator.[8] Therefore, we also tested the sensitivity of peripheral blood cells to regulation by the glucocorticoid agonist dexamethasone and the β2-adrenergic receptor

agonist terbutaline. It has been well established that glucocorticoid receptor agonists will inhibit the proliferative response of T cells.[9-11] Terbutaline is known to inhibit TNF-α production and to enhance IL-10 production by peripheral blood cells in healthy individuals.[11;12]

Methods

The Medical Research Ethics Committee of the St. Antonius Hospital Nieuwegein, the Netherlands approved the study protocol, MEC ID: R-06.38A, GOV ID: NL14786.100.06.

Study population

One hundred ninety-three sarcoidosis patients (irrespective of the presence of complaints of fatigue) were invited to participate in the study. They were selected from a database of 800 sarcoidosis patients and selection criteria were: sarcoidosis had been diagnosed according to the latest ATS/ERS/WASOG statement on sarcoidosis[1]; probability to meet the in- and exclusion criteria (see Table 1) on the basis of available medical records. Out of 193 patients, 88 were eligible for screening. After signing the informed consent, they were tested for in- and exclusion criteria. The inclusion criteria focus on the clinical remission of sarcoidosis, while the exclusion criteria rule out other causes and disabilities which might also explain the presence of fatigue.

Thirteen out of 88 patients were excluded after screening because of: signs of active disease (increased serum sIL-2R, ACE, calcium or CRP; n=8); Löfgren's syndrome as disease onset (n=2); thyroid disorder (n=1); diabetes (n=1) and one patient judged participation as too demanding. In total, 75 patients were included. Blood sampling failed in 4 patients (3 fatigued and 1 non-fatigued).

Table 1 In- and exclusion criteria

Inclusion criteria:	
Previously involved organs	no clinical signs of disease activity
Serum parameters (sIL-2R, ACE, Ca^{2+}, CRP)	normal
Chest X-ray	normal or 2 years or more stable
Lung function	normal or 2 years or more stable, i.e.:
VC or FEV_1	<10% change
DLco	<15% change
Exclusion criteria:	
Löfgren's syndrome	at onset of sarcoidosis
Use of corticosteroids or other immunosuppressive drugs	over the last 6 months
Use of analgesic medication	over the last 3 months
Antidepressive medication	over the last 3 months
Psychiatric diseases (patient-reported)	
Sleep apnea, narcolepsy or restless legs syndrome (by history)	
Hb, Cell Blood Count, Na^{2+}, K^{+}, creatinine, GGT, AF, ASAT, ALAT, LDH, glucose, total protein, TSH, ferritine, routine urine analysis	abnormal
BMI	<17 or ≥35
Abuse of alcohol, drugs or other substance (by anamneses)	
Age	>65 years

sIL-2R = soluble interleukin-2 receptor, ACE = angiotensin converting enzyme, Ca^{2+} = calcium, CRP = C-reactive protein, VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity of the lung for carbon monoxide, BMI = body mass index (kg/m^2)

Questionnaires

Fatigue was measured by self report with the Checklist Individual Strength (CIS).[13] A cut-off of 35 on the subscale *fatigue-severity* was used to allocate patients to the fatigued or the non-fatigued group.

Psychological distress was assessed with the Symptom Checklist 90 (SCL-90)[14;15], a self-report, multidimensional list of 90 psychological symptoms. The total score on this questionnaire reflects the degree of actually experienced psychological distress.

Trier Social Stress Test

The Trier Social Stress Test (TSST) is a standardized laboratory test which avoids interindividual variances and induces reliable cortisol and adrenocorticotropic hormone (ACTH) levels as well as norepinephrine and epinephrine increases in serum, in response to psychosocial stress.[7;16] All experimental sessions commenced between 10:00 AM and 10:30 AM and lasted for approximately 2 hours. Patients were instructed to take a light meal on the morning of testing and refrain from caffeinated beverages, chocolate, tropic fruit(juice), nuts, tomatoes, alcohol and aspirin.

Forty minutes before the first blood sample, an intravenous catheter was inserted. Next, the patient was attached to a Monitoring System (Philips M4 M3046A) that registered heart frequency and blood pressure with intervals of 10 minutes until end of second resting period. After an initial resting period (watching a relaxing video) of 30 minutes, the patient was transferred to the test room. Here the patient was exposed to the TSST: a free speech (mock job interview during 5 minutes) and a mental arithmetic task (serial subtractions, also during 5 minutes) in front of a videocamera. Before the stress task, the patient received a short instruction to the speech task, followed by a period of 2 minutes of preparation. Only when performing the stress tasks, the patient was in an upright position. Once both tasks were completed, the patient was reseated and debriefed during 10 minutes. Next, the patient was returned to the first room, followed by a second rest period of 30 minutes (and a second relaxing video).

Blood sampling and biochemical analysis

In total 6 blood samples were taken: (1) after initial rest period, (2) after transfer to test room, (3) after instruction, (4) after stress tasks, (5) after debriefing, and (6) after second rest period. Blood samples for the in vitro sensitivity of peripheral blood cells for dexamethasone and terbutaline were part of the first blood sample.

Blood samples were collected into pre-chilled ethylene diamine tetra acetic acid (EDTA) tubes and kept on ice. Glutathione was added to the blood samples to optimize stabilization of norepinephrine and epinephrine. Plasma was separated within 30 minutes after sampling and stored at -80 °C until analyzed.

ACTH was determined using a solid-phase, two-site sequential chemiluminescent immunometric assay (Immulite 2500, Siemens Healthcare Diagnostics). Cortisol was determined using a solid-phase, competitive chemiluminescent enzyme immunoassay for cortisol (Immulite 2500, Siemens Healthcare Diagnostics). Norepinephrine and epinephrine were measured with High-Performance Liquid Chromatography (HPLC) with electrochemical detection (ECD) as described.[17]

Receptor sensitivity tests

In vitro sensitivity of peripheral blood cells for dexamethasone

Whole blood was diluted 1:10 in medium [RPMI 1640 (Gibco, Grand Island, NY) supplemented with 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin]. Diluted blood (100 μL) was cultured for 96 h in round bottom 96-well plates (Nunc, Glostrup, Denmark) with 25 μL phytohemagglutinin (PHA) (HA 15; Murex Diagnostics, Dartford, UK), final concentration 25 μg/mL, and 25μL DEX in the concentrations indicated. At 16–18 h before the end of the culture, 1 μCi (37 kBq) [^{3}H]-thymidine was added. At the end of the culture period, cells were harvested by the use of an automated cell harvester and were counted as cells per

minute. Incorporated radioactivity was determined in a liquid scintillation counter. Results are presented in percentages inhibition of T cell proliferation.

In vitro sensitivity of peripheral blood cells for terbutaline

Whole blood was diluted 1:10 in medium, and 100 μL diluted blood was cultured with 50 μL LPS [Escherichia coli (DIFCO Laboratories, Detroit, MI); final concentration 2 ng/mL] and 50 μL medium or the β2-adrenergic receptor agonist terbutaline (Sigma Chemical Co., St. Louis, MO). After 18 h of culture at 37 °C, supernatants were harvested and stored at -80 °C until analysis. TNF-α and IL-10 levels were determined by enzyme-linked immuosorbent assay (Pelikine; CLB, Amsterdam, the Netherlands). Results are presented as percentages decrease in TNF-α, respectively increase in IL-10 as compared to the level of TNF-α, respectively IL-10 in cultures without terbutaline.

Statistical analysis

Data on ACTH, cortisol, epinephrine and norepinephrine were all log transformed to approximate the normality assumptions. As gender can have important effects on responsiveness to the TSST[16], all analyses were conducted for females and males separately. In addition, smoking and use of contraception were introduced as covariates, as these factors may also influence neuroendocrine outcome of the response to the TSST. As this study involved repeated measurements, the analysis was carried out with the Linear Mixed Models repeated measurement approach in SPSS version 16.0.

Data on demographic variables and other patient characteristics were analyzed with one way ANOVA and Chi-square tests. In all analyses, the significance level was set at $p < 0.05$.

Results

Study population

Seventy-one patients out of a cohort of 75 were included in the present study: blood sampling failed in 4 patients. All patients were free from disease activity. Median time since diagnosis was 10 years, with interquartile range of 5 to 18 years.

According to the standardized cut-off of the *fatigue-severity* subscale of the CIS[13], patients who scored ≥35 were assigned to the fatigued group (n=34); all other patients were assigned to the non-fatigued group (n=37). Characteristics of demography and medical history of both groups are summarized in Table 2. In females as well as in males no significant differences were found between fatigued and non-fatigued subjects with respect to age, BMI, demographics, smoking, time since diagnosis, and medical history. However, total scores of the SCL-90 were significantly higher in the fatigued group compared to the non-fatigued group in males as well as females.

Neuroendocrine responses to TSST

Norepinephrine

The TSST elicited in both females and males significant responses of norepinephrine over time, which did not differ between the non-fatigued group and the fatigued group (Table 3, Figure 1a and 1b).

Table 2 Characteristics of the study group

	Females			Males		
	Non-fatigued	Fatigued	p	Non-fatigued	Fatigued	p
Number	16	23		21	11	
Fatigue severity (CIS-subscale score) median [IQR]	16 [12-20]	46 [37-50]		14 [10-23]	43 [37-46]	
Age (mean ± SD)	46 ± 6	46 ± 8	0.694	48 ± 8	46 ± 9	0.368
Body Mass Index (kg/m^2)	24 ± 3	25 ± 4	0.316	26 ± 2	25 ± 5	0.75
Marital status			0.638			0.786
single	2	2		2	0	
married/cohabitant	14	19		18	10	
divorced	0	2		1	1	
Highest level of education			0.614			0.283
primary	0	2		0	0	
secondary	6	5		5	1	
upper secondary	7	8		7	5	
tertiary/higher	3	8		9	5	
Smoking			0.811			0.784
yes	2	3		3	3	
former	6	6		7	3	
never	8	14		11	5	
Median time since diagnosis [IQR] (in years)	10 [3.25-18]	8 [5-17]	0.928	11 [6-18.5]	13 [6-21]	0.877
History of corticosteroids						
yes	3	8	0.471	10	5	0.906
if used: median period of use in months [IQR]	10 [8-12]	12 [4-37]	0.405	23 [18-31]	10 [6-24]	0.485
Localistation at diagnosis			0.678			0.775
pulmonary	14	18		18	9	
extrapulmonary	2	5		3	2	
Total score SCL-90 mean ± SD	114 ± 36	139 ± 27	**0.021**	112 ± 16	135 ± 41	**0.029**

Table 3 Neuroendocrine and cardiovascular responses to TSST

	Time effect	Group Effect	Time by Group Effect
Norepinephrine			
females	F = 50.43, $p < 0.0001$	F = 0.04, $p = 0.83$	F = 2.00, $p = 0.08$
males	F = 65.72, $p < 0.0001$	F = 0.002, $p = 0.96$	F = 0.75, $p = 0.58$
Epinephrine			
females	F = 6.28, $p < 0.0001$	F = 0.39, $p = 0.53$	F = 3.34, $p =$ **0.007**
males	F = 10.71, $p < 0.0001$	F = 3.68, $p = 0.06$	F = 1.05, $p = 0.39$
ACTH			
females	F = 7.16, $p < 0.0001$	F = 0.83, $p = 0.36$	F = 0.41, $p = 0.83$
males	F = 31.36, $p < 0.0001$	F = 1.84, $p = 0.18$	F = 0.66, $p = 0.65$
Cortisol			
females	F = 5.82, $p < 0.0001$	F = 0.08, $p = 0.78$	F = 1.85, $p = 0.11$
males	F = 31.25, $p < 0.0001$	F = 3.73, $p = 0.06$	F = 0.38, $p = 0.86$
Heart rate			
females	F = 23.02, $p < 0.0001$	F = 0.06, $p = 0.80$	F = 0.47, $p = 0.75$
males	F = 24.82, $p < 0.0001$	F = 0.007, $p = 0.93$	F = 0.62, $p = 0.64$
Systolic blood pressure			
females	F = 8.77, $p < 0.0001$	F = 10.59, $p =$ **0.001**	F = 0.17, $p = 0.94$
males	F = 19.84, $p < 0.0001$	F = 0.98, $p = 0.32$	F = 0.28, $p = 0.88$
Diastolic blood pressure			
females	F = 8.17, $p < 0.0001$	F = 19.42, $p <$ **0.0001**	F = 0.11, $p = 0.98$
males	F = 12.06, $p < 0.0001$	F = 1.57, $p = 0.21$	F = 0.17, $p = 0.95$

Epinephrine

In both females and males, the TSST induced a significant response over time. In females a significant interaction effect (time by group effect, p = 0.007) was observed. Further analysis showed that the increase in epinephrine was absent in fatigued females, while the stress-induced epinephrine concentrations of the non-fatigued female patients peaked after completing the stress tasks (Figure 2a). In fatigued males we did observe a stress-induced rise in epinephrine levels, but epinephrine levels peaked already during the anticipatory phase and remained stable during the actual stress task, whereas the non-fatigued group showed a rise in epinephrine at the expected time point, i.e. in response to the performance of the stress tasks (Figure 2b).

In addition, the fatigued males tended to have overall lower epinephrine concentrations compared to non-fatigued males (p = 0.06). Post-hoc testing showed significantly lower pre-test epinephrine levels in fatigued males (p = 0.036).

Figure 1 Changes in norepinephrine in response to the TSST. Graphs show the mean and the SEM of the fatigued (—) and the non-fatigued (---) group for (a) females and (b) males.

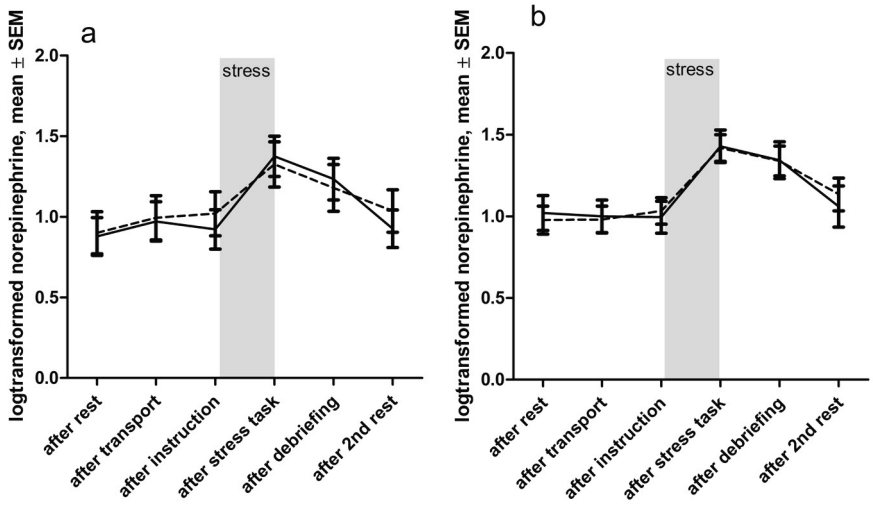
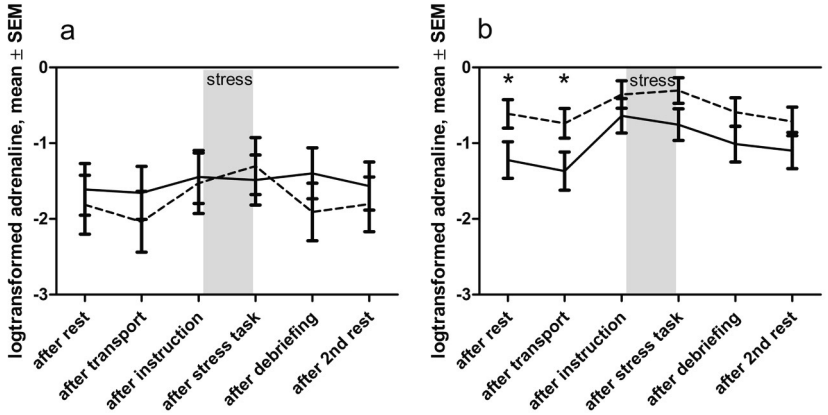


Figure 2 Changes in epinephrine in response to the TSST. Graphs show the mean and the SEM of the fatigued (—) and the non-fatigued (---) group for (a) females and (b) males.



ACTH and cortisol

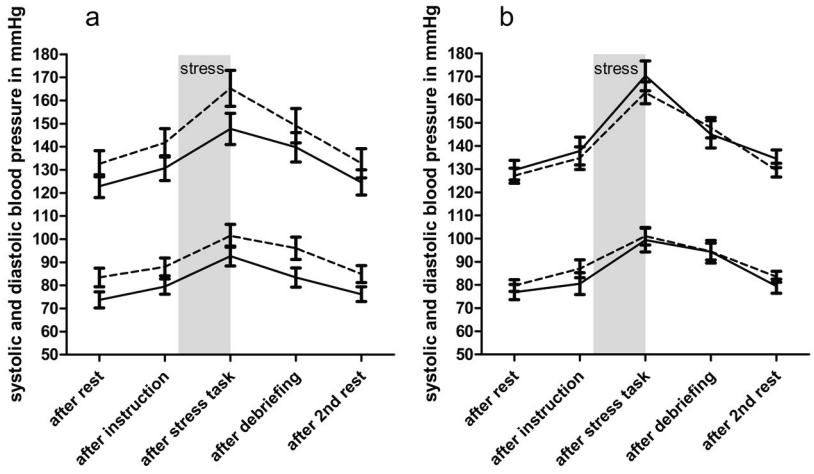
Significant ACTH and cortisol responses were induced by the TSST in both females and males over time. The responses of ACTH and cortisol did not differ between the non-fatigued group and the fatigued group in both genders.

Cardiovascular response

Significant systolic and diastolic blood pressure responses were provoked by the TSST over time in females as well as in males. The fatigued females had a lower systolic and diastolic blood pressure over the whole test period than the non-fatigued females (Figure 3a). The fatigued and non-fatigued males did not differ with respect to stress-induced responses in systolic and diastolic blood pressure (Figure 3b).

The TSST generated significant heart rate responses in females and males over time, but no group differences or interaction effects were observed.

Figure 3 Changes in systolic and diastolic blood pressure in response to the TSST.
Graphs show the mean and the SEM of the fatigued (—) and the non-fatigued (---) group for (a) females and (b) males.



Receptor sensitivity of peripheral blood cells

Dexamethasone

The synthetic glucocorticoid dexamethasone induced a significant inhibition of T cell proliferation by peripheral blood cells of females as well as of males of the fatigued group as well as of the non-fatigued group, but the inhibitory activity of dexamethasone did not differ between the non-fatigued group and the fatigued group (Table 4).

Table 4 Results on receptor sensitivity tests

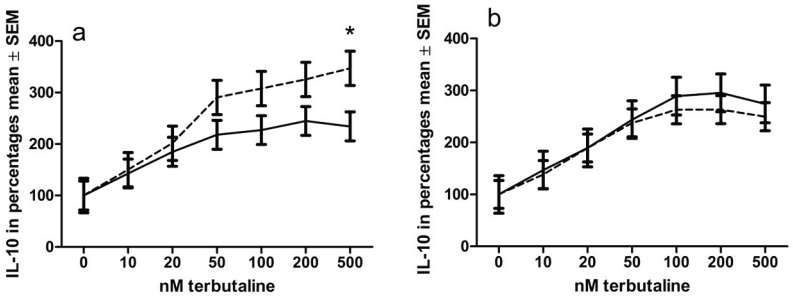
	Time effect	Group Effect	Time by Group Effect
Dexamethason			
females	F = 659.76, $p < 0.0001$	F = 0.29, $p = 0.59$	F = 0.16, $p = 0.99$
males	F = 698.55, $p < 0.0001$	F = 1.78, $p = 0.19$	F = 0.67, $p = 0.69$
TNF-α			
females	F = 96.44, $p < 0.0001$	F = 0.46, $p = 0.49$	F = 0.43, $p = 0.85$
males	F = 96.61, $p < 0.0001$	F = 0.46, $p = 0.50$	F = 0.73, $p = 0.62$
IL-10			
females	F = 10.49, $p < 0.0001$	F = 2.18, $p = 0.14$	F = 2.08, $p = 0.06$
males	F = 9.68, $p < 0.0001$	F = 1.54, $p = 0.69$	F = 0.41, $p = 0.87$

Terbutaline

Terbutaline inhibited TNF-α production and increased IL-10 production of LPS-stimulated peripheral blood cells of both females and males. Peripheral blood cells of the fatigued females showed a trend to lower IL-10 production (p = 0.068) and post-hoc tests revealed a significant difference in IL-10 production at the concentration of 500 nMol terbutaline (p = 0.011, Figure 4). In males the IL-10 production of LPS-stimulated peripheral blood cells did not differ between the non-fatigued group and the fatigued group.

No differences were observed between the non-fatigued group and the fatigued group for TNF-α production by peripheral blood cells of both genders (Table 4).

Figure 4 β2-adrenergic regulation of IL-10 production. Data are presented in percentage of IL-10 levels in the absence of terbutaline, and represent the mean and SEM. Graphs show the mean and the SEM of the fatigued (—) and the non-fatigued (---) group for (a) females and (b) males.



Discussion

This is the first study that investigated the difference in responsiveness of the sympathetic nervous system and the hypothalamus pituitary adrenal axis to an acute stressor as well as the receptor sensitivity of peripheral blood cells for endocrine signalling in patients clinically recovered from the inflammatory disease sarcoidosis.
We tested 71 sarcoidosis patients in clinical remission, of whom 48% reported severe fatigue 10 years after diagnosis of the disease, with the Trier Social Stress Test (TSST). Our study showed a blunted epinephrine secretion in response to the stressor in fatigued sarcoidosis patients in clinical remission when challenged with the TSST. Compared to non-fatigued individuals, stress-induced responses of epinephrine were characterized as inadequate in both fatigued females as fatigued males. Moreover, fatigued males showed a trend towards overall lower epinephrine levels over the whole test period in comparison to non-fatigued males. Furthermore, fatigued females showed lower systolic and diastolic blood pressure compared to non-fatigued females. Finally, peripheral blood cells of fatigued female patients were less reactive to β2-adrenergic stimulation with respect to induction of IL-10.
The finding of a dysregulated stress-induced epinephrine response in chronically fatigued sarcoidosis patients is novel. However, we did observe an increase in norepinephrine in response to the stressor in these chronically fatigued patients. The cause of the latter discrepancy between norepinephrine and epinephrine is unknown, but may be caused by a selective dysregulation of the regulatory pathways involved in epinephrine synthesis/ secretion by the adrenal medulla, or dysregulated direct neuronal activation.[18] Furthermore, physical exercise is known to increase epinephrine secretion and sympathetic output.[19-21] The physical inactivity of the fatigued patients may therefore be an explanation for an attenuated epinephrine response. In a previous study [3] we showed a significant reduction of physical activity levels objectively measured with an accelerometer as well as muscle weakness (respiratory muscles, handgrip and quadriceps) in our fatigued patient group. In our opinion, physical inactivity may therefore well be responsible for the lower epinephrine responses in the chronically fatigued sarcoidosis patients in clinical remission.

In addition, fatigued males had significantly lower basal epinephrine levels compared to non-fatigued males. However, comparison with the norm score values for this epinephrine test revealed that the baseline epinephrine concentrations in the non-fatigued males were extremely high and above norm scores: 0.63 ± 0.109 nmol/l; 0.17 nmol/l was the upper limit of the norm value. Nevertheless, basal epinephrine concentrations of the other three groups were higher than the norm score values as well: non-fatigued females 0.338 ± 0.12 nmol/l, fatigued females 0.389 ± 0.07 nmol/l and fatigued males 0.35 ± 0.08 nmol/l. These increased basal epinephrine values may be attributed to anticipatory stress of the test day. However,

in our experience, using this test in experiments with other patient groups, we never found increased catecholamine levels.[22;23] The high basal epinephrine levels might be the cause of a low reactivity of epinephrine to the stressor, since the output of the system may already be maximal or even exhausted. Remarkably, the basal norepinephrine levels were within the range of norm score values, so the discrepancy between epinephrine and norepinephrine is also apparent on the level of the basal plasma concentrations. The basal cortisol and ACTH plasma levels were also within norm score values. Therefore we are inclined to suggest that the increased basal epinephrine levels are not the result of a general basal arousal of the stress system of the fatigued patients, but due to a selective dysregulation of epinephrine output.

In another study we investigated whether the prolonged fatigue after clinical remission of sarcoidosis was associated with changes in the immune responses. We assessed cytokine/chemokine profiles of peripheral blood leukocytes of the patients in the same study group. We showed that chronically fatigued sarcoidosis patients in clinical remission had less capacity to produce Th2 cytokines than non-fatigued patients in remission. In sarcoidosis the first phase is characterized by a profound Th1 pattern which shifts to a Th2 dominated pattern during the phase after granuloma formation. This shift is thought to be beneficial as it restores the Th1/Th2 balance thereby limiting the ongoing inflammation. An incapability to raise an adequate Th2 response as we observed in our previous study, might contribute to the persistence of chronic fatigue.[24]

The Th1/Th2 balance is also regulated by the sympathetic nervous system.[6;25;26] Catecholamines, especially epinephrine, downregulate Th1 cytokines such as IL-12, TNF-α and IFN-γ and upregulate Th2 cytokine production such as IL-10 and transforming growth factor-β. In the present study we have observed an attenuated response of epinephrine to acute psychosocial stress in all fatigued patients. Therefore, we would like to propose that the impaired epinephrine response to the stressor might contribute to the low Th2 immune response. The latter theory is further supported by the fact that the receptor for epinephrine on peripheral blood leukocytes of female fatigue patients is less sensitive to stimulation by epinephrine as shown by a lower responsiveness to the β2-adrenergic agonist terbutaline. The non-responsiveness of epinephrine levels to the stressor would imply that there may be less daily fluctuations in epinephrine in combination with a low sensitivity of the receptor for epinephrine.

In conclusion, the present study shows an attenuated response of the sympathetic nervous system to acute psychosocial stress in chronically fatigued sarcoidosis patients in clinical remission in combination with a decreased sensitivity of the immune system for adrenergic stimulation in fatigued females. The cause of the selective dysregulation remains to be determined, but physical inactivity and deconditioning may be important in this respect.

We propose that an inability to mount a reactive epinephrine response to daily stressors may increase the risk of decreased anti-inflammatory immune functioning. Since regular physical exercise may normalize sympathetic output, we are inclined to recommend graded exercise therapy as to improve the neuroendocrine response to stress and the health status of chronically fatigued sarcoidosis patients in clinical remission significantly.

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5

Attenuated Laser Evoked Potentials in chronically fatigued sarcoidosis patients in clinical remission

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Abstract

Pain and fatigue are the most commonly reported symptoms in sarcoidosis. Even when features of the granulomatous inflammation have resolved and the disease is in clinical remission, pain complaints persevere particularly in patients who also suffer from persisting fatigue. We hypothesized that pain in these chronically fatigued patients may be related to dysfunctioning of the Aδ nociceptive processing which is responsible for transporting pain signals.

Laser Evoked Potentials were recorded in patients with sarcoidosis in clinical remission reporting chronic fatigue (n=30), a non-fatigued group (n=32) and a group of healthy volunteers (n=28). Electroencephalographic recordings following laser stimulation were evaluated in terms of area under the curve (AUC), latencies (N2, P2) and N2P2-amplitudes. Evoked pain was evaluated with Numeric Rating Scale (NRS). In addition, general pain complaints were evaluated by MacGill Pain Questionnaire.

Chronically fatigued patients in clinical remission showed significantly smaller AUCs, N2P2-amplitudes, and NRS on stimulus-intensity 2.0 Watt compared to healthy controls. No significant differences between groups were found on the LEP latencies. Evaluation of general pain complaints in daily life revealed that chronically fatigued patients reported significant more pain points ($p = 0.011$), including pain in hands and feet ($p = 0.002$) compared to non-fatigued subjects and to healthy controls.

This is the first study reporting that Aδ nociceptive processing is reduced in chronically fatigued patients in which clinical signs of sarcoidosis have resolved, compared to healthy controls. The latter data might explain the observed elevated levels of general pain complaints in daily life in these patients.

Introduction

Pain and fatigue are the most often reported symptoms in sarcoidosis.[1-4] Sarcoidosis is a systemic inflammatory granulomatous disorder of unknown cause which regularly resolves within 2 to 4 years.[5] From clinical reports[6;7] and expert experience in our clinic, it is known that pain and fatigue complaints may persist even when features of disease activity have resolved. In a previous study[8] we showed that 49% of our study group that consisted of sarcoidosis patients with disease in clinical remission suffered from persisting fatigue, up to 9 years after diagnosis. This chronic fatigue appeared to be significantly associated with pain complaints.
A possible explanation for the persistence of these pain complaints may be found in the reports on reduction of intraepidermal nerve fiber densities (IENFD) in sarcoidosis patients. Studies on skin biopsies[9-11] showed significantly lower intraepidermal nerve fiber densities in sarcoidosis patients compared to healthy volunteers. So, loss of small fibers in the skin has been demonstrated in sarcoidosis patients with active disease. On the basis of these data, we hypothesized that pain symptoms in chronically fatigued patients with sarcoidosis *in clinical remission* are possibly related to dysfunction of the small fibers as well. Hence, we tested the Aδ nociceptive processing with a method that is considerably less invasive than skin biopsy and which is regularly applied for diagnostic and research purposes in our clinic[12]: laser evoked potentials (LEP).
In summary, the aim of this study was to investigate the hypothesis that Aδ nociceptive processing is reduced in chronically fatigued patients with sarcoidosis in clinical remission in comparison with healthy controls.

Methods

Subjects

One hundred ninety-three sarcoidosis patients (irrespective of the presence of complaints of fatigue or pain) were invited to participate in the study. They were selected from a database of 800 sarcoidosis patients and selection criteria were: sarcoidosis had been diagnosed according to the latest ATS/ERS/WASOG statement on sarcoidosis[5]; probability to meet the in- and exclusion criteria (see Table 1) on the basis of available medical records. Upon signing the informed consent, they were tested for in- and exclusion criteria (listed in Table 1). After screening, 62 participants were included in this study. Based on the standardized cut-off on the *fatigue-severity* subscale of the CIS[13], patients with a score ≥35 were allocated to the 'fatigued group'; the others to the 'non-fatigued group').
In addition, data on laser evoked potentials of 28 healthy volunteers, recruited from hospital personnel, were used as a healthy control group. These data had already been sampled

before the start of the present study. Finally, we like to emphasize that none of the participants received analgesic treatment at the time of the study.
The Medical Research Ethics Committee of the St. Antonius Hospital Nieuwegein, the Netherlands approved the study protocol, MEC ID: R-06.38A, GOV ID: NL14786.100.06.

Laser and stimulation protocol

Cutaneous heat stimuli were delivered by a 980-nm diode laser (Biolitec, Ceram Optec, Bonn, Germany) to the blackened dorsum of the right foot. Blackening of the skin was performed to rule out bias by differences in skin pigmentation. Laser intensity varied between 1.0 and 2.0 W (10–20 mJ/mm^2), duration of the stimuli was set at 50 msec, with a laser beam spotsize of 5 mm^2. The stimulus intensity would evoke a clear pinprick sensation. Interstimulus duration randomly varied between 5 and 10 seconds to assure reorientation of attention to an unexpected stimulus.
The stimulation protocol comprised 6 runs of each 10 stimuli, in the following sequence: 1.0 Watt, 2.0 Watt, 1.5 Watt, 1.5 Watt, 2.0 Watt, and 1.0 Watt. In order to minimize skin irradiation and nociceptor habituation the laser target was slightly displaced after each stimulation. Participants and experimenters wore protective goggles throughout the experiment.

Table 1 In- and exclusion criteria

Inclusion criteria:	
Previously involved organs	no clinical signs of disease activity
Serum parameters (sIL-2R, ACE, Ca^{2+}, CRP)	normal
Chest X-ray	normal or 2 years or more stable
Lung function	normal or 2 years or more stable, i.e.:
VC or FEV_1	<10% change
DLco	<15% change
Exclusion criteria:	
Löfgren's syndrome	at onset of sarcoidosis
Use of corticosteroids or other immunosuppressive drugs	over the last 6 months
Use of analgesic medication	over the last 3 months
Antidepressive medication	over the last 3 months
Psychiatric diseases (patient-reported)	
Sleep apnea, narcolepsy or restless legs syndrome (by history)	
Hb, thrombocytes, leucocytes, Na^{2+}, K^+, creatinine, GGT, AF, ASAT, ALAT, LDH, gluc, protein electrophoresis, TSH, ferritine, routine urine analysis	abnormal
BMI	<17 or ≥35
Abuse of alcohol, drugs or other substance (by anamneses)	
Age	>65 years

sIL-2R = soluble interleukin-2 receptor, ACE = angiotensin converting enzyme, Ca^{2+}= calcium, CRP = C-reactive protein, VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity of the lung for carbon monoxide, BMI = body mass index (kg/m^2)

Recording and processing

Electroencephalographic (EEG) recordings were made to measure brain activity following laser stimulation. Silver disk electrodes were positioned on the skull according to the International 10–20 system. Cz, C3, C4, and Pz electrodes from the vertex were linked to both ear lobes (A1, A2). Impedance was kept below 5 kOhm. Electrooculography was recorded for eye movement artifact filtering. Online single sweep EEG monitoring was performed. Recordings showing blinks, eye movements, or any other artifacts were manually deleted followed by offline response averaging and analysis. EEG signals were sampled at 1000 Hz after band-pass filtering (0.5–30 Hz) by Viasys Healthcare Inc. (Viasys Healthcare Inc., Houten, the Netherlands). EEG epochs were recorded between 200 msec before until 800 msec after the onset of each stimulus.

Evaluation of evoked pain

Subjective evaluation of pain evoked by laser stimulation was quantified with a Numeric Rating Scale (NRS). Before the stimulation protocol started a single reference laser stimulus of 1.0 Watt was administered corresponding to a NRS of 4. Then the protocol started and after each run the participants were asked to rate the evoked pain between 0 and 10.

Evaluation of fatigue and general pain complaints

Self-reported fatigue was measured with the Checklist Individual Strength (CIS).[13] A cut-off of 35 on the subscale *fatigue-severity* was used to identify a fatigued and a non-fatigued group. General pain complaints in daily life were assessed with MacGill Pain Questionnaire (MPQ) – Dutch Version.[14;15] The subscales *Number of reported pain points, Time since pain started*, *Type of pain*, and *Quality of Life* were used in this study.

The healthy control group did not fill out both questionnaires as this group had already been sampled before the start of this study.

Statistical analysis

Each stimulus-intensity was applied twice. The mean area under the curve (AUC) (μV/msec), mean N2P2-amplitude (μV), and mean latencies (msec) were computed for every participant per stimulus-intensity by offline averaging of corresponding stimulation blocks.

NRS per stimulus intensity was calculated by averaging the ratings of corresponding runs.

As LEP recordings were repeated measurements, the analysis was carried out with the Linear Mixed Models repeated measurement approach in SPSS version 16.0. All data resulting from LEP recordings were logtransformed. Data on demographic variables and questionnaires scores were analyzed with one way ANOVA and Chi-square tests. In all analyses, the significance level was set at $p < 0.05$.

Results

Study population

Sixty-two patients with histopathologically proven sarcoidosis were included. In all patients no disease activity could be detected: serum parameters (ACE, sIL-2R, CRP, CA^{2+}) were normal; lung function was within normal range. In the past, at the time of diagnosis, 10 patients had had extrapulmonary localisation of sarcoidosis, but physical examination at time of study revealed no clinical signs of disease activity in any organs anymore. One (fatigued) patient presented with slight bihilair lympadenopathy; chest X-ray revealed no abnormalities in all other patients. Median time since diagnosis was 9.5 years, with interquartile range of 5.7 to 17 years.

Table 2 Population characteristics, including overview of evaluation of general pain complaints in daily life by means of McGill Pain Questionnaire (only filled out by the non-fatigued and the fatigued sarcoidosis patients in clinical remission)

	Healthy controls	**Non-fatigued**	**Fatigued**	p value
Number	28	32	30	
Gender				0.231
male	13	18	10	
female	15	14	20	
Age in years (mean ± SD)	34.79 ± 8.03	47.81 ± 7.9	47.06 ± 8.89	**<0.001**
Time since diagnosis (median [IQR])		8 [4.25-17]	10.5 [6.7-18.2]	0.411
Fatigue severity (CIS-subscale score, mean ± SD)		17.94 ± 7.22	44.63 ± 6.58	
Former use of corticosteroids				
yes		13	10	0.431
months of use (median [IQR])		21 [16-31]	11 [4-23]	0.711
Number of pain points (median [IQR])		1 [0-2]	3 [1-5.5]	**0.011**
Report on pain in:				**0.002**
hands		5	2	
feet		0	5	
hands and feet		0	5	
Type of pain				**0.016**
no pain		13	3	
single episode, limited duration		11	9	
continuous, fluctuating severity		6	15	
continuous, nonfluctuating severity		2	3	
Time since start pain (yrs) (median [IQR])		1 [0-3.75]	7 [2-16]	**0.001**
Quality of Life		10.94 ± 2.77	14.52 ± 5.67	**0.005**

According to the standardized cut-off on the *fatigue-severity* subscale of the CIS, 30 patients were allocated to the 'fatigued group' and 32 patients to the 'non-fatigued group'. In addition, data of 28 healthy controls were used as control group in this study.

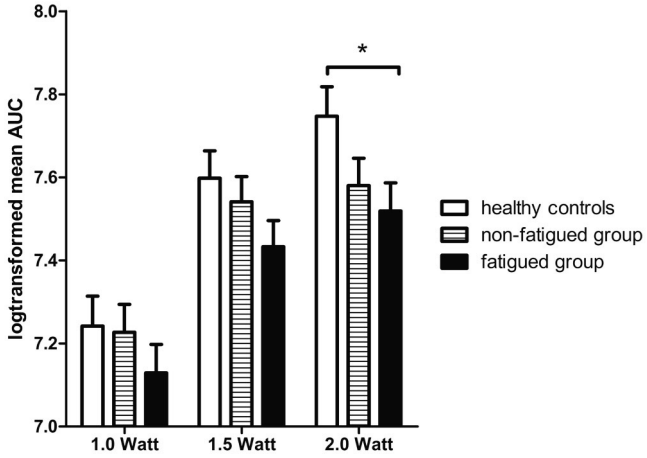
Characteristics of the 3 groups, including an overview of pain complaints in daily life of the patients are summarized in Table 2. No differences were found between groups with respect to gender, time since diagnosis, and history of corticosteroid use, except for age: the group of healthy controls was significantly younger ($p < 0.001$). Therefore, the effect of age was tested in all statistical analyses. Differences were also found with respect to general pain complaints in daily life. The fatigued group reported significantly more pain points compared to non-fatigued group ($p = 0.011$), including more reports on pain in hands and feet ($p = 0.002$). The fatigued group had also been suffering significantly longer from pain ($p = 0.001$) and had higher scores on measurements of quality of life ($p = 0.005$). The type of pain that is characterized as 'Continuous, fluctuating severity' was most frequently reported in the fatigued group ($p = 0.016$).

Laser Evoked Potentials

AUC

Analysis revealed that the increase of stimulus-intensity elicited a significant increase of the AUC ($F(2, 100) = 63.965$, $p < 0.0001$), irrespective of group membership. The between group comparison as well as the interaction between stimulus-intensity and group membership turned out to be non-significant ($F(2, 86) = 2.19$, $p = 0.118$ resp. $F(4, 100) = 0.752$, $p = 0.559$). Age did not significantly contribute to the increase of the AUC ($F(50, 35) = 0.768$, $p = 0.808$). Post hoc pairwise comparison showed that the AUC on stimulus-intensity 2.0 Watt was significantly smaller in the fatigued group compared to the controls ($p = 0.022$). In the non-fatigued group the AUC on stimulus intensity 2.0 Watt was also smaller compared to controls, but this difference was not significant ($p = 0.086$). Also no significant differences in AUC on stimulus-intensity 2.0 Watt was observed between the fatigued and the non-fatigued group ($p = 0.523$, Figure 1).

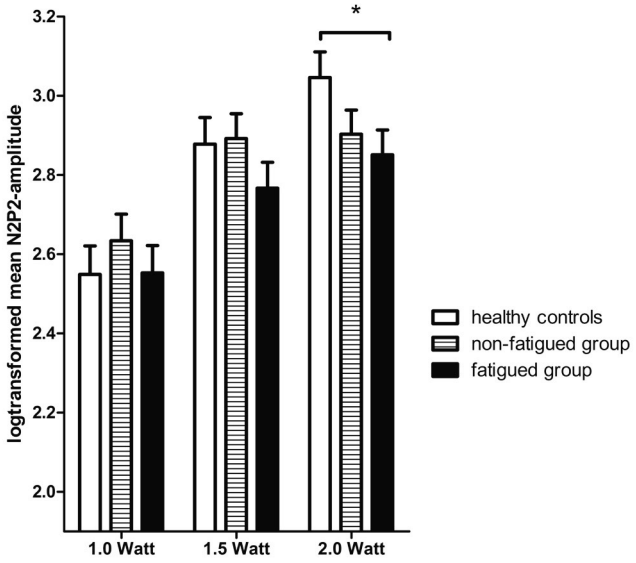
Figure 1 Mean ± SEM logtransformed area's under the curve (AUC) on 3 laser intensities in healthy controls, non-fatigued patients with sarcoidosis in clinical remission, and fatigued patients with sarcoidosis in clinical remission (* $p < 0.05$ healthy controls versus fatigued group).



N2P2-amplitudes

The latter pattern was also present in N2P2-amplitudes: a significant effect of stimulus-intensity ($F(2, 104) = 42.940$, $p < 0.0001$), but none related to the between group comparison ($F(2, 87) = 1.024$, $p = 0.363$) or the interaction between stimulus-intensity and group membership ($F(4, 104) = 1.863$, $p = 0.122$). Age was not a significant contributor to the increase of N2P2-amplitudes $F(50, 35) = 0.731$, $p = 0.848$). Post hoc pairwise comparison showed that the N2P2-amplitudes on stimulus-intensity 2.0 Watt were significantly smaller in the fatigued group than in controls ($p = 0.034$). Also the non-fatigued group displayed on stimulus intensity 2.0 Watt lower N2P2-amplitudes compared to controls, but this difference was not significant ($p = 0.111$). Again no significant differences in N2P2-amplitudes on stimulus-intensity 2.0 Watt were observed between the fatigued and the non-fatigued group ($p = 0.56$, Figure 2).

Figure 2 Mean ± SEM logtransformed N2P2-amplitudes on 3 laser intensities in healthy controls, non-fatigued patients with sarcoidosis in clinical remission, and fatigued patients with sarcoidosis in clinical remission (* $p < 0.05$ healthy controls versus fatigued group).



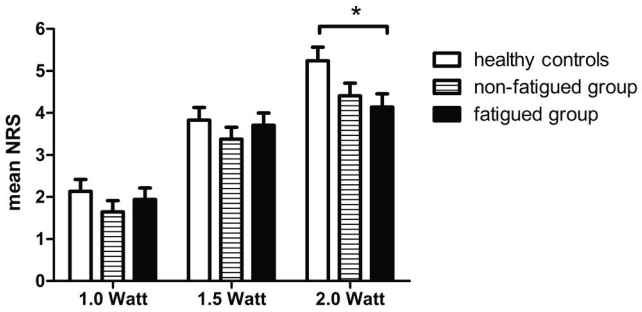
N2- and P2-latencies

The analysis showed no significant differences between the 3 groups on the LEP latencies (N2, P2). Age did not significantly contribute to the increase of LEP latencies.

NRS

The increase of stimulus-intensity elicited a significant increase of Numeric Ratings ($F(2, 89) = 230.530$, $p < 0.0001$), irrespective of group membership. Both the between group comparison ($F(2, 80) = 1.390$, $p = 0.255$), as well as the interaction between stimulus-intensity and group membership ($F(4, 89) = 2{,}226$, $p = 0.073$) turned out to be non-significant. Also, the contribution of age to the increase of NRS was not significant $F(50, 34) = 0.896$, $p = 0.645$). However, post-hoc pairwise comparisons showed significant lower ratings in the fatigued group compared to the controls at stimulus intensity 2.0 Watt ($p = 0.016$). Also the non-fatigued group showed lower NRS scores than the control group at stimulus intensity 2.0 Watt, but this difference was again not significant ($p = 0.063$). Finally, no significant differences in NRS on stimulus-intensity 2.0 Watt were observed between the fatigued and the non-fatigued group ($p = 0.542$, Figure 3).

Figure 3 Mean ± SEM Numeric Rating Scale scores on 3 laser intensities in healthy controls, non-fatigued patients with sarcoidosis in clinical remission, and fatigued patients with sarcoidosis in clinical remission (* $p < 0.05$ healthy controls versus fatigued group).



Discussion

This is the first study that investigates pain processing via small (Aδ-) fibers in sarcoidosis patients in clinical remission of the disease. We are the first to show that, compared to healthy controls, Aδ nociceptive processing is significantly reduced in fatigued sarcoidosis patients in clinical remission.

Both AUCs, N2P2-amplitudes, and NRS scores on stimulus-intensity 2.0 Watt were significantly smaller in fatigued patients compared to healthy controls. Smaller AUCs and N2P2-amplitudes in response to LEP point to a dysfunction of the nociceptive processing of Aδ fibers. This dysfunction may be present anywhere in the sensory pathway from the skin to the cortex. Latency data (N2 and P2 latencies) did not differ between the 3 groups, suggesting a normal conduction time for ascending volleys reaching the cortex following laser stimulation of the skin. Therefore, a demyelinating lesion in those pathways is not very likely. Considering the fact that in sarcoidosis a loss of intraepidermal nerve fibers has been demonstrated using skin biopsy[10;11], the smaller AUCs and N2P2-amplitudes in the fatigued group may well be attributed to this phenomenon. An additional positive argument for this assumption is the recent report of a strong relationship between LEP and intraepidermal nerve fiber density.[16] We therefore propose that the attenuated *LEP* in fatigued sarcoidosis patients in clinical remission may be the result of axonal loss of the peripheral fibers in the skin. From this point of view the lower NRS scores at 2.0 Watt which we found in the fatigued group correspond well with the smaller AUCs and N2P2-amplitudes: as a consequence of fiber loss the fatigued group experiences less pain, especially at a high stimulus-intensity.

Interestingly, this study showed as well that AUCs and N2P2-amplitudes in the non-fatigued group were lower than those of controls, although these differences did not reach statistical significance. One may therefore put forward that the preceding disease (i.c. sarcoidosis) may have triggered alterations in the nociceptive processing to different extents in both patient groups. According to the literature, immune-mediated diseases[17;18] may induce damage to small fibers. For sarcoidosis is a systemic inflammatory disorder which is immunologically characterized by elevated levels of interleukin (IL)-2, interferon (IFN)-γ, and subsequently macrophage tumor necrosis factor (TNF)-α[19-21], it is likely that the cause of the neuropathy may be attributed to this inflammation. Nevertheless, more research is needed to definitely prove the mechanism of perpetuating deteriorated nociceptive processing particularly in fatigued patients when signs of inflammations have long resolved. In this light, one may even speculate whether the disease status of the sarcoidosis patients is convincingly enough 'in remission'. As no golden standard for 'the clinical remission state' of sarcoidosis is available, we did the best we could to exclude patients with active disease in this study. Nevertheless, although clinical signs of disease activity lack, our patients still suffered from chronic fatigue and pain complaints which might be a more indicative and sensitive sign for lingering disease activity than laboratory, lung function or chest X-ray tests. We would therefore like to hypothesize that a subclinical, low-grade inflammatory activity could be well responsible for the observed dysfunction of small fibers.
Furthermore, we like to remark that the mean age of the healthy control group was significantly lower than the mean age of both patient groups. IEFND is known to be age and gender related: lower densities were found in men and in older age.[10;22] The reduced LEP-AUCs and latencies which we found in the fatigued group could then possibly be an effect of age. Therefore, the contribution of age was determined in all analyses. Age did not show a significant effect on the increase of AUCs, N2P2-amplitudes and NRSscores. Therefore we conclude that age has not affected our study results.

Finally, evaluation of general pain complaints in daily life revealed elevated levels in the fatigued group as compared to the non-fatigued group. This observation seems to contradict the results on reduced NRS scores in response to evoked pain. However, from literature it is known that LEP can be attenuated when the stimulus is administered in painful territories, even in patients suffering from hyperalgesia or allodynia.[23] Since the fatigued group reported more daily complaints of pain in hand and feet, this could well explain their lower AUCs and N2P2-amplitudes.
We therefore propose that pain complaints in daily life may be attributed as neuropathic pain which may arise as a direct consequence of the Aδ dysfunction we observed in this study.

This is the first study that investigates pain processing via small (Aδ-) fibers in sarcoidosis patients in clinical remission of the disease. We are the first to show that, compared to healthy controls, Aδ nociceptive processing is significantly reduced in chronically fatigued sarcoidosis patients in clinical remission. This might explain the observed elevated levels of general pain complaints in daily life in this fatigued group.

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6

Post-inflammatory fatigue in sarcoidosis: personality profiles, psychological symptoms and stress hormones

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submitted

Abstract

Objectives *Chronic fatigue following inflammatory diseases has been well documented. However, little is known about the possible risk factors of chronic post-inflammatory fatigue. The aim of this study was to investigate whether chronic post-inflammatory fatigue after clinical remission of the disease sarcoidosis is associated with specific dimensions of personality, psychological symptoms and baseline levels of stress hormones.*

Methods *Thirty-seven non-fatigued and 33 fatigued patients in clinical remission of sarcoidosis were evaluated with the Temperament and Character Inventory- short form (TCI); the Symptom Check List-90 (SCL), and the Checklist Individual Strength (CIS). Baseline levels of ACTH and cortisol were measured in plasma. Principal component analysis with orthogonal rotation (varimax) was conducted on all personality, psychological and stress hormone data in order to obtain a smaller set of components. Logistic regression analysis was performed to associate these components with chronic post-inflammatory fatigue.*

Results *Principal component analysis identified 5 components, of which two components were significantly associated with chronic post-inflammatory fatigue. The first component comprised the personality trait Harm Avoidance and all SCL-subscales except Sleep. The second component consisted of baseline levels ACTH and cortisol, and showed an inverse association with chronic post-inflammatory fatigue. The 3 other identified components, consisting of respectively [1] TCI-Cooperativeness and SCL-Sleep, [2] TCI-Novelty Seeking/Reward Dependence/Self Transcendence, and [3] TCI-Persistence, were not significantly associated with chronic fatigue.*

Conclusion *Chronic post-inflammatory fatigue after clinical remission of sarcoidosis is associated with a triad of risk factors: a specific personality profile with profound neurotic characteristics in combination with high levels of psychological distress, and decreased baseline ACTH/cortisol levels.*

Introduction

Chronic post-inflammatory fatigue is a well-known debilitating symptom that can even persist when an infection or inflammatory process has resided. The literature gives ample evidence of chronic fatigue syndromes after infections with viruses like Epstein-Barr virus[1], Ross River virus[2], Coxiella burnetii[3;4], and enterovirus.[5;6] But also non-infectious diseases have been associated with chronic fatigue such as rheumatic arthritis[7], cancer[8;9], or Crohn's disease.[10] From all these reports it is obvious that, irrespective of the type of infection or inflammation, patients report – besides fatigue – the same constellation of fatigue-related symptoms: pain, muscular weakness, poor mental concentration, and increased and long-lasting malaise after exertion.

In a recent study[11] we showed that an analogous pattern of complaints was found in patients clinically recovered from the inflammatory disease sarcoidosis. Sarcoidosis is a systemic granulomatous disorder which is immunologically characterized by the presence of CD4+ T cells in the alveoli of the lungs as well as in peripheral blood. Activated CD4+ T cells differentiate into type 1 helper (Th1) cells secreting predominantly interleukin (IL)-2, interferon (IFN)-γ and macrophage tumor necrosis factor (TNF)-α production. Th1 cells also stimulate macrophages to the production of TNF-α and IFN-γ, thus creating a cytokine-loop. The complex of this immune response contributes to the development and accumulation of granulomas which is the hallmark of the disease. Granuloma formation may involve any organ, but is often manifested in the lymphatic system and lungs. Sarcoidosis has no known cause; it commonly affects young and middle-aged adults. Usually, sarcoidosis resolves within 2 to 4 years.[12-14] Besides functional problems concerning the affected organs, patients suffer during the active phase of the disease from a range of non-specific conditions such as fatigue, fever, arthralgia, and depressive mood.[15]
Particularly complaints of fatigue may persist, when clinically no disease-activity can be detected anymore. In a previous study we showed that this fatigue was present in 49% of our study population which consisted of sarcoidosis patients with disease in clinical remission. In these patients chronic fatigue had persisted even up to 9 years after diagnosis and resulted in substantial deterioration of health status. In 47% of cases the fatigue profile met the internationally accepted criteria for Chronic Fatigue Syndrome[16], of note to mention that sarcoidosis was acknowledged as precipitating trigger for fatigue.[11]

Recent studies on personality characteristics in patients diagnosed with Chronic Fatigue Syndrome (CFS) gave evidence for associations between personality profiles and chronic fatigue.[17] In particular, increased levels of neuroticism and perfectionism[17-21] seem to be a consistent outcome in CFS patients. Neuroticism and perfectionism are both regarded as

heritable tendencies that manifest as automatic pre-conceptual responses in early life. The concepts of neuroticism and perfectionism emerge in Cloninger's personality model as: Harm Avoidance and Persistence.[22;23] Harm Avoidance involves the tendency to respond intensely to signals of aversive stimuli, thereby inhibiting behavior. It is observed as pessimistic worry in anticipation of problems, fear of uncertainty, shyness with strangers, and rapid fatigability. People high in Harm Avoidance are fearful, socially inhibited, shy, passive, easily tired, and pessimistic even in situations that do not worry other people.
Persistence is defined as the tendency to persevere in behaviors that have been previously associated with reward or relief from punishment, despite frustration and fatigue. It is observed as industriousness, determination, and perfectionism. Highly Persistent people are hard-working, perseverant, and ambitious overachievers who tend to intensify their effort in response to anticipated reward and perceive frustration and fatigue as a personal challenge.[24] Some personality characters seems to be strongly correlated with psychological distress.[25;26] For example, the interrelation of depression and anxiety disorders with Harm Avoidance has been confirmed in several studies.[27-34]

Fatigue in CFS patients has also often been studied in relation to the activity of the hypothalamus pituitary adrenal (HPA) axis. There is evidence of hypocortisolism (as measured in plasma, salvia and urine) and support for blunted adrenocorticotropic hormone (ACTH) responses in challenge tests in patients diagnosed with CFS.[35-37] However, literature on the association between the activity of the HPA axis and post-inflammatory or post-infection fatigue has only been reported for chronic fatigue after Epstein-Barr virus infection[38] and in quiescent inflammatory bowel disease.[10]

Until now, it is unclear which risk factors contribute particularly to chronic post-inflammatory fatigue. In the present study we examined in a group of fatigued and non-fatigued sarcoidosis patient who were in clinical remission whether personality traits, psychological symptoms and baseline stress hormones (cortisol and ACTH) were associated with chronic post-inflammatory fatigue.

Methods

This study was approved by the Medical Research Ethics Committee of the St. Antonius Hospital Nieuwegein, the Netherlands, MEC ID: R-06.38A, GOV ID: NL14786.100.06.

Participants

One hundred ninety-three sarcoidosis patients were invited to participate in the study either during their visit at the outpatient clinic of the St. Antonius Hospital Nieuwegein, or by written request from their former treating pulmonary physician. They were selected from a database of 800 sarcoidosis patients irrespective of the presence of complaints of fatigue. Selection criteria were: diagnosis of sarcoidosis according to the latest ATS/ERS/WASOG statement on sarcoidosis[12]; probability to meet the in- and exclusion criteria (see Table 1) on the basis of available medical records. Out of 193 patients, 115 responded to the invitation, and 88 of them were eligible for screening. After signing the informed consent, they were tested for in- and exclusion criteria. The in- and exclusion criteria are listed in Table 1. We emphasize that patients with diagnosed psychiatric diseases or who had been receiving psychiatric medication over the past 3 month were excluded from participation.

After screening, 75 (out of 88) participants were included. Thirteen participants were excluded because of: signs of active disease (increased serum sIL-2R or ACE or calcium) (8); Löfgren's syndrome as disease onset (2); thyroid disorder (1); diabetes (1) and one patient judged participation as too demanding.

Table 1 In- and exclusion criteria

Inclusion criteria:	
Previously involved organs	no clinical signs of disease activity
Serum parameters (sIL-2R, ACE, Ca^{2+}, CRP)	normal
Chest X-ray	normal or ≥ 2 years stable
Lung function tests:	normal or ≥ 2 years stable:
VC or FEV_1	<10% change
DLco	<15% change
Exclusion criteria:	
Löfgren's syndrome	at onset of sarcoidosis
Use of corticosteroids or other immunosuppressive drugs	over the last 6 months
Antidepressive medication	over the last 3 months
Psychiatric diseases (patient-reported)	
Sleep apnea, narcolepsy or restless legs syndrome (by anamneses)	
Hb, blood cell count, Na^{2+}, K^{+}, creatinin, GGT, AF, ASAT, ALAT, LDH, glucose, total protein, TSH, ferritin, routine urine analysis	abnormal
BMI	<17 or ≥35
Abuse of alcohol, drugs or other substance	
Age	>65 years

sIL-2R = soluble interleukin-2 receptor, ACE = angiotensin converting enzyme, Ca^{2+}= calcium, CRP = C-reactive protein, VC = vital capacity, FEV_1 = forced expired volume in the first second of expiration, DLco = diffusion capacity of the lung for carbon monoxide, BMI = body mass index (kg/m^2)

Measurements

Checklist Individual Strength (CIS)

Self-reported fatigue was measured with the Checklist Individual Strength (CIS).[39] The CIS possesses excellent psychometric properties concerning internal reliability and discriminant validity. The questionnaire consists of 20 statements, scored on a 7-point Likert scale ("yes, that is true" – "no, that is not true"), and includes four subscales: fatigue-severity; concentration; motivation; and activity. A total sum results in a multidimensional total fatigue score. A cut-off of 35 on the subscale *fatigue-severity* was used to identify a fatigued and a non-fatigued group.

Temperament and Character Inventory-short form (TCI)

For the assessment of Cloninger's dimensions of personality we used the Dutch version of the Temperament and Character Inventory-short form (TCI).[24;40] The validity of this version has previously been confirmed.[41] The TCI is a self-report questionnaire which comprises 105 statements scored on a dichotomous scale ("correct" – "incorrect"). According to Cloninger's model, 4 dimensions of temperament are assessed (Novelty Seeking, Harm Avoidance, Reward Dependence, and Persistence), and 3 dimensions of character (Self-Directedness, Cooperativeness, and Self-Transcendence).

Symptom Checklist-90 (SCL)

The Symptom Checklist-90[42;43] is a self-report, multidimensional list of 90 psychological symptoms. Respondents are asked to report on a 5-point Likert scale ranging from 0 ("not at all") to 4 ("extremely") the extent to which they have experienced 90 different psychological symptoms within the past 7 days. The questionnaire consists of the 8 subscales: Depression, Obsessive-Compulsive, Anxiety, Phobic Anxiety, Sleep, Somatisation, Interpersonal Sensitivity, and Hostility.

Blood sampling

Blood samples were collected after 30 minutes of rest in a relaxing chair via an intravenous catheter, centrifuged at 1800 x G for 10 minutes at 4 °C, and plasma was stored at -80 °C. ACTH and cortisol were determined using a solid-phase, two-site sequential chemiluminescent immunometric assay for ACTH and a solid-phase, competitive chemiluminescent enzyme immunoassay for cortisol (Immulite 2500, Siemens Healthcare Diagnostics).

Statistical analysis

Data analyses were performed using Statistical Package for the Social Sciences version 16.0 (SPSS 16.0). Prior to analysis all variables were tested for normality (Levene's test). After that, baseline ACTH and cortisol concentrations underwent natural logtransformation.

We aimed to bundle the effect of correlation between personality characteristics, psychological symptoms and stress hormones by running a principal component analysis. In order to attain the best set of interpretable components, a varimax rotation (an orthogonal rotation method that keeps components unrelated) was used. Only components with eigenvalues >1 were selected. Loadings ≥0.6 were used to identify the variables comprising a component. Next, logistic regression analysis (enter method) was used to assess the contribution of the selected components as a predictor for chronic post-inflammatory fatigue.

Results

Characteristics of the study group

Seventy-five sarcoidosis patients in state of clinical remission of the disease were included in this study. Two patients refused to fill out the TCI (both fatigued) and in three patients blood sampling failed (1 non-fatigued; 2 fatigued). Data of 70 patients were analyzed.

In all patients no signs of disease activity could be detected: serum parameters and lung function tests were normal. Physical examination at the time of the study revealed no clinical signs of disease activity. Radiographic examination showed two participants with slight bihilair lymphadenopathy (1 fatigued; 1 non-fatigued); no abnormalities were found in all other participants. Median time since diagnosis was 9 years, with interquartile range of 5 to 17.

The total study group was split in two groups according to the standard cut-off for severe fatigue on the subscale *fatigue-severity* of the CIS (≥35)[39]: a non-fatigued group (n=37) and a fatigued group (n=33). Characteristics of both groups are summarized in Table 2. No differences were found between both groups with respect to gender, age, BMI, or demographics.

Table 2 Characteristics of the non-fatigued and fatigued group (split was based on the cut-off (≥35) on the subscale fatigue-severity of the CIS)

	Non-fatigued group	**Fatigued group**	p value
Number	37	33	
Fatigue severity (CISsubscale score) min - max	16.76 ± 6.66 8 - 31	44.42 ± 6.48 35 - 56	
Gender			0.050
male	21	11	
female	16	22	
Age min - max	47;10 ± 7;45 32.08 – 61.41	46;8 ± 8;11 30.41 – 65.66	0.534
BMI	25.40 ± 2.87	25.62 ± 4.56	0.815
Marital status			0.459
single	4 (10.8%)	2 (6.1%)	
married	32 (86.5%)	28 (84.8%)	
divorced	1 (2.7%)	3 (9.1%)	
Year of diagnosis (median [IQR])	1997 [IQR: 1990-2003]	1997 [IQR: 1990-2002]	0.90
Education			0.373
primary	0 (0%)	2 (6.1%)	
secondary	11 (29.7%)	6 (18.2%)	
upper secondary	14 (37.8%)	13 (39.4%)	
tertiary/higher	12 (32.4%)	12 (36.4%)	

Principal component analysis

In order to bundle the effect of correlation between the personality, psychological and hormonal data, these were clustered into components by principal component analysis. This analysis resulted in the extraction of 5 components with eigenvalues >1 that explained together 69.42% of the total variance of all data. Table 3 shows that the first component comprised a constellation of factors consisting of 1 personality trait (TCI-Harm Avoidance) and 7 psychological symptoms (SCL-Anxiety, -Phobic Anxiety, -Depression, -Somatisation, -Obsessive-Compulsive, -Interpersonal Sensitivity, -Hostility). Therefore this component was labeled as 'Psychoneurotic distress'-cluster. Component 2 yielded the personality trait Cooperativeness and the psychological symptom Sleep and was named 'Cooperativeness'. Component 3 contained a cluster of personality characteristics: TCI-Novelty Seeking, -Reward Dependence, -Self Transcendence. This cluster was referred to as 'Novelty Seeking'. Baseline levels of ACTH and cortisol loaded high on component 4 which we characterize as 'HPA axis'-cluster. The personality trait 'Persistence' formed component 5 that was labeled as such.

Table 3 Summary of principal component analysis of subscales of TCI, subscales of SCL and logtransformed ACTH and logtransformed cortisol in 70 sarcoidosis patients in clinical remission, rotated component loadings ≥ 0.6 in bold

			Rotated	**Component**	**Loadings**
			Components		
	1	2	3	4	5
TCI subscales					
Novelty Seeking	-.183	.248	**.778**	-.231	-.027
Harm Avoidance	**.774**	-.072	-.227	.092	-.283
Reward Dependence	.029	-.502	**.659**	.155	-.232
Persistence	-.107	-.044	.053	.060	**.835**
Self Directedness	-.537	-.434	-.198	.091	.347
Cooperativeness	-.208	**-.804**	.041	-.141	.001
Self-transcedence	.290	.019	**.642**	-.008	.181
SCL subscales					
Anxiety	**.781**	.348	.148	-.100	.096
Phobic anxiety	**.763**	.013	-.167	.052	.075
Depression	**.758**	.245	.205	-.022	-.267
Somatisation	**.635**	.442	.110	-.178	-.273
Obsessive-compulsive	**.680**	.095	.113	-.198	-.350
Interpersonal sensitivity	**.693**	.395	.048	-.014	-.076
Hostility	**.809**	.162	.171	-.074	.304
Sleep	.194	**.631**	.075	-.007	-.053
Baseline stress hormones					
ACTH	-.024	-.055	-.166	**.814**	.076
cortisol	-.103	.126	.069	**.899**	.007
Eigenvalue	5.891	1.882	1.466	1.386	1.177
% of variance	34.65	11.06	8.62	8.15	6.92
Label of component	Psycho neurotic distress	Co operativeness	Novelty Seeking	HPA axis	Persist-ence

Logistic regression analysis

The five selected components ('Psychoneurotic distress', 'Cooperativeness', 'Novelty Seeking', 'HPA axis' and 'Persistence') were fed into a logistic regression analysis to assess their association with chronic post-inflammatory fatigue (Table 4). Significant power to predict fatigue was found in the 'Psychoneurotic distress'-cluster ($p = 0.005$) and the 'HPA axis'-cluster ($p = 0.024$). The latter cluster appeared to be inversely associated.

Table 4 Results on logistic regression analysis

	B (SE)	95%CI for Odds Ratio		p value
		Lower	Upper	
Psychoneurotic distress	1.279 (0.455)	1.472	8.765	**0.005**
Sleep	0.306 (0.289)	0.771	2.393	0.289
Novelty Seeking	0.055 (0.286)	0.603	1.851	0.847
HPA axis	-0.698 (0.309)	0.271	0.912	**0.024**
Persistence	-0.408 (0.297)	0.372	1.191	0.170

Discussion

This study shows a significant association between the personality dimension Harm Avoidance, psychological distress and decreased baseline levels of ACTH/cortisol and post-inflammatory chronic fatigue of patients in clinical remission of sarcoidosis.

High scores on Harm Avoidance indicate that chronically fatigued patients with sarcoidosis in clinical remission seem to be more cautious, careful, fearful, insecure or pessimistic, even in situations that generally do not worry other people. High Harm Avoidance has not only been reported in the healthy population (where this factor is considered as a predictor of fatigue-related disorders[30;44]), but also in patients diagnosed with CFS.[18;19] Interestingly, in CFS also increased scores on the Persistence-scale have been found.[18;19] However, in our study population of sarcoidosis patients in clinical remission Persistence was not associated with chronic post-inflammatory fatigue. Therefore we conclude that the personality profile in chronic fatigue after inflammation is different from that reported for CFS.

Moreover, the 'Psychoneurotic distress'-cluster which was identified by the principal component analysis consisted of the personality trait Harm Avoidance and 7 out of 8 psychological symptoms. The existence of this so-called 'Psychoneurotic distress'-cluster is in line with literature reporting that high scores on Harm Avoidance are strongly associated with depression and anxiety. Nevertheless, we cannot conclude how these factors may have influenced each other over time, since data on both psychological symptoms and personality profiles at the time before/at the onset of, and during the inflammation are not available.
However, personality is generally judged as trait that is heritable and fairly stable over time.[45-48] In addition, in another inflammatory disease, rheumatoid arthritis, personality characters of neuroticism proved to be the most consistent and effective predictor for long term psychological distress, irrespective of initial distress levels, biomedical factors, use of medication and other stressors or vulnerability factors.[49] Although that study concerned patients with *active* inflammatory disease, one may hypothesize that elevated levels of neuroticism may contribute

to maintaining fatigue after resolution of the inflammation as well. This hypothesis is supported by the results of personality studies in cancer survivors which showed that the personality trait neuroticism was significantly associated with quality of life and mental morbidities such as fatigue at long term follow up.[50;51]

Also the 'HPA axis'-cluster that was identified by the principal component analysis showed significant predictive power for chronic post-inflammatory fatigue. This component was inversely associated with chronic fatigue which suggests that fatigued patients in clinical remission have lower baseline levels of ACTH/cortisol compared to the non-fatigued group. Alterations in the HPA axis, including mild hypocortisolism, increased negative feedback or blunted ACTH responses to challenge tests (administration of HPA-hormones, psychological stress or physical exercise) have frequently been reported in patients diagnosed with CFS.[35] However, prospective studies suggest that HPA axis changes are absent during the early stages of illness, and that the observed changes may develop somehow during the course of the illness, leading to the hypothesis that changes in the activity of the HPA axis may occur as a consequence of the illness and is in principal triggered by inactivity and deconditioning.[52]

From our previous study[11], we know that physical inactivity (as objectively measured with an accelerometer) and muscle weakness (respiratory muscles, handgrip and quadriceps) are significantly associated with chronic fatigue in our study group. The observed reduction of ACTH/cortisol baseline values could therefore be probably the result of inactivity. However, the low baseline levels of ACTH/cortisol may also be related to a neuroendocrine dysregulation, either as a result of the initial inflammatory process or resulting from a persisting low-grade inflammation during the phase of 'clinical remission'. In this respect it is of interest that preliminary studies of our group indicate that post-inflammatory fatigue after sarcoidosis is associated with a decreased capacity to produce anti-inflammatory cytokines during the remission phase.

In chronic post-inflammatory fatigue it is not known yet whether administration of cortisol is an option to resolve this vicious circle. Nevertheless, we suggest a combination of graded exercise and psychological therapy possibly supplemented with temporary low dose cortisol replacement as an approach to counteract the long-lasting debilitating symptoms of post-inflammatory fatigue.

In summary, we show here that chronic post-inflammatory fatigue after clinical remission of sarcoidosis is associated with a specific personality profile with profound neurotic characteristics in combination with high levels of psychological distress, and decreased baseline ACTH/cortisol levels. This triad of risk factors may be taken into account by psychotherapists treating the symptoms of chronic post-inflammatory fatigue.

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7

Summary and general discussion

Follow up

Future remarks

Characteristics of post-inflammatory fatigue in sarcoidosis

When the inflammatory signs of sarcoidosis have resolved, chronic fatigue may persist in a substantial group of sarcoidosis patients. In our study group 49% of the patients remained chronically fatigued. Median time since diagnosis was 9 years. Fatigued sarcoidosis patients suffered from severe and disabling fatigue that had started at the onset of sarcoidosis. This post-inflammatory fatigue was accompanied by a range of fatigue-related symptoms: concentration problems, pain (sore throat, muscle, headache, multi-joint, lymph nodes), unrefreshing sleep, and pronounced feelings of malaise and longer recovery time after exertion. Moreover, compared to non-fatigued patients, chronically fatigued patients with sarcoidosis in clinical remission revealed more psychological symptoms like depression and anxiety, lower health status, and reduced muscle strength. Scores on self-reported sleep time did not differ between fatigued and non-fatigued participants. Furthermore, comparison with norm scores of a healthy control group learned that mean physical activity levels as measured with an accelerometer were significantly reduced in chronically fatigued sarcoidosis patients in clinical remission.

Psycho-biological characteristics

Apart from characterizing the symptoms of fatigue in sarcoidosis, this thesis also focussed on psychological and biological factors possibly associated with this chronic post-inflammatory fatigue in sarcoidosis patients. We concentrated on: cytokine/chemokine profiles; the neuroendocrine system and response to a stressor; nociception by peripheral nerve fibers; and personality and psychological factors.

Cytokine/chemokine profiles

In this thesis we showed that chronic post-inflammatory fatigue in sarcoidosis is associated with a specific cytokine/chemokine profile. The capacity to produce Th2 cytokines (IL-4, IL-5 and IL-10) in vitro was decreased in chronically fatigued patients with sarcoidosis in clinical remission compared to non-fatigued patients in clinical remission. Furthermore, we observed higher levels of LPS-induced IL-8 production, as well as lower levels of plasma MCP-1 in the chronically fatigued group in comparison with the non-fatigued patients in clinical remission.

The observed reduced capacity to produce Th2 cytokines in vitro in chronically fatigued patients with sarcoidosis in clinical remission led to the hypothesis that these fatigued patients were less able to make the shift from a Th1 to a Th2 response which is part of the immune response counterbalancing the Th1 cytokine production during the course of sarcoidosis. The

acute phase of sarcoidosis is characterized by a profound Th1 activity which switches to a Th2 dominated pattern of activity in the phase after granuloma formation. Taken together, we would like to speculate that the incapability to raise an adequate Th2 response may also contribute to the persistence of chronic fatigue after the clinical signs of disease activity have resolved.
From this point of view we might even question the conclusion that the fatigued sarcoidosis patients in our study group are truly in clinical remission. Although physical examination, serum tests, imaging techniques, and lung function tests did not reveal any signs of disease activity, it may be hypothesized that the observed reduced capability to produce Th2 cytokines is in fact a sign of low-grade immune dysregulation. Moreover, in this respect it is worth mentioning that in fatigued patients the LPS-induced production of IL-8 was increased and plasma levels of MCP-1 were decreased. In this light, we propose that the lack of Th2 cytokine production in vitro is in fact the most sensitive sign of residual disease activity in sarcoidosis.

Apart from the 'Th2 cytokines', the principal component analysis identified (in chapter 3) also a cluster of 'Th1 cytokines'. The cytokine TNF-α loaded high on this component. The latter is of interest, since TNF-α plays a key role in the active phase of sarcoidosis, and effective treatment of severe refractory sarcoidosis consists of TNF-α blockers like infliximab.[1-4] Treatment with infliximab in 45 refractionary sarcoidosis patients in our hospital significantly improved lung function, downregulated inflammation markers in serum, but also decreased fatigue severity scores significantly.[5] From these results we may suggest that TNF-α might be an important inducer of fatigue in the active phase of disease.
Although we do not know whether the mechanism that maintains chronic fatigue *after clinical remission* of the disease is the same as in active disease, and even though the positive association between the 'Th1 cytokines' component and chronic fatigue did not reach statistical significance ($p = 0.078$), it is not unlikely that TNF-α might contribute to generating feelings of fatigue in the clinical remission phase as well.

It is now well established that the Th1/Th2 balance is influenced by glucocorticoids and catecholamines.[6-8] Glucocorticoids as well as catecholamines inhibit the production of IL-12, IFN-γ, and TNF-α, and upregulate the production of IL-4, IL-10, and IL-13. Through this mechanism increased levels of glucocorticoids and/or catecholamines may systemically cause a selective suppression of the Th1 activity in combination with a shift toward Th2 mediated immunity.[9] Now that we have observed a reduced capacity to produce Th2 cytokines in vitro in the chronically fatigued subgroup of our study population, one might assume that glucocorticoids may be involved and may be downregulated in these patients as well. However, comparison of baseline levels of cortisol between the fatigued and the non-fatigued group (in chapter 4) did not reveal a significant difference, even when corrected for gender.

Additionally, we like to mention that these baseline cortisol levels of the fatigued as well as the non-fatigued group were within normal score values. Moreover, the sensitivity of glucocorticoid receptors on peripheral blood cells did not differ between fatigued and non-fatigued patients. We might therefore propose that neither basal levels of glucocorticoids nor the sensitivity of the glucocorticoid receptor did account for the reduced levels of Th2 cytokines production in chronically fatigued patients in our study.
Furthermore, we investigated whether catecholamines may have contributed to the reduced capacity to produce Th2 cytokines. In males we observed lower basal epinephrine levels in the fatigued group compared to the non-fatigued group. Closer analysis revealed that basal epinephrine levels of both non-fatigued males and females, as well as their fatigued counterparts were higher than the norm score values. Moreover, in response to an acute stressor the expected epinephrine increase was absent in fatigued patients (females as well as males) (see chapter 4 and discussed below). Since sympathetic activation occurs frequently during daily life thereby participating in the 'setpoint' of many immune responses[10-12], we are allowed to speculate that a low or absent non-reactive stress-induced epinephrine output might partially explain the low Th2 basal immune response.

Neuroendocrine response to stress

In response to acute psychosocial stress the HPA axis did not differ between the fatigued and the non-fatigued group. Therefore we would like to suggest that this result supports the view that glucocorticoids are not responsible for the reduction in the capability to produce Th2 cytokines.
Remarkably, our data show that catecholamines could explain the reduced Th2 cytokines. In fatigued patients epinephrine levels did not show the expected response to an acute psychological stressor. Moreover, it is of interest that we found that peripheral blood cells of female fatigued patients were less sensitive to regulation by β2-adrenergic agonist terbutaline with respect to induction of the Th2 cytokine IL-10 as compared to non-fatigued female patients. From these data the picture emerges that fatigued sarcoidosis patients in clinical remission have a decreased adrenergic response to acute stress, which may be intensified in fatigued females by a reduced sensitivity of the immune system to adrenergic stimulation. This constellation may (at least partially) explain the reduced capacity to produce Th2 cytokines.

The absence of an epinephrine response to acute stress is remarkable, because the norepinephrine concentrations did significantly change in response to the stressor.
A dissociation between responses in norepinephrine and epinephrine in response to an acute stressor like the TSST has not been described before in relation to an inflammatory disease. A possible explanation for this dissociation may be a reduced capability to convert epinephrine from norepinephrine (by the enzyme Phenylethanolamine N-methyltransferase,

that is produced in the chromafin cells of the adrenal medulla). An other explanation for this dissociation may be found in reduced levels of physical activity. As physical exercise is known to increase epinephrine secretion and sympathetic output[13-16], the observed muscle weakness and reduced physical activity levels in the fatigued individuals of our study group may be well responsible for a decreased epinephrine response. From these observations we are inclined to recommend graded exercise therapy to chronically fatigued sarcoidosis patients in clinical remission. Not only in order to improve their physical functioning, but also to treat dysregulation of the sympathetic nervous system and restore the capacity to produce Th2 cytokines.

Nociception by peripheral nerve fibers

As it is known that active sarcoidosis patients who complain of burning pain in hands and feet, may be affected by a loss of small fibers[17-19], the presence of high levels of daily experienced pain in the fatigued subgroup of our study cohort, and especially the presence of burning pain in hands and feet, urged us to further investigate this phenomenon.

Pain processing via small (Aδ-) fibers was examined in sarcoidosis patients in clinical remission of the disease and in healthy controls by means of laser evoked potentials (LEP). Aδ nociceptive processing was significantly reduced in chronically fatigued sarcoidosis patients in clinical remission, pointing to a dysfunction of the nociception of Aδ fibers. This dysfunction may be present anywhere in the lateral, sensory pathway from the skin to the cortex. As in active sarcoidosis a loss of intraepidermal nerve fibers has been demonstrated using skin biopsy[17-19] and as a strong relationship between LEP and intraepidermal nerve fiber density[20] has been established, the smaller AUCs and N2P2-latencies in the fatigued group may well be attributed to intraepidermal nerve fiber loss.

Immune-mediated diseases may induce damage to small fibers.[21;22] Since sarcoidosis is a systemic inflammatory disorder, we are tempted to propose that the alterations in the Aδ nociceptive processing may have been induced by sarcoidosis during the active phase of the disease. Our study also showed lower laser evoked AUCs and N2P2-amplitudes in the non-fatigued group compared to controls, even though these differences did not reach statistical significance. From this observation, we would like to raise the hypothesis that sarcoidosis might affect small fibers in any case in all sarcoidosis patients. Subsequently, the damage of these Aδ fibers might then persist after resolution of the inflammation in different degrees which is reflected in the increase in pain complaints which are associated with chronic post-inflammatory fatigue.

Although it is widely accepted that damage to the Aδ fibers may result in neuropathic pain[23;24], we are not sure whether the impaired nociception by small fibers is the sole cause of the elevated pain scores of the chronically fatigued group. Actually, the fatigued group reported more frequently burning pain in hands and feet, but also suffered more often from pain

in knees, head, neck, shoulders, and lower back. These types of pain complaints are not classified as neuropathic pain. In addition, we have to take into account that the fatigued group reported significantly more often than the non-fatigued patients that the pain was 'continuously present, and fluctuating in severity'. Moreover, the fatigued group indicated that this pain had been present for more than 7 years. All together, these observations point to the presence of a condition that may be defined as 'chronic pain'.[25] For this reason we should recognize that other factors than damage to the Aδ fibers may contribute to the elevated pain scores of the chronically fatigued sarcoidosis patients as well. We might then think of sensitization of the central nociceptive system. Persistent nociceptive input from peripheral lesions due to impaired Aδ fibers which are processed by the lateral nociceptive system, may lead to increased neuronal responsiveness or sensitization of the cental nociceptive system. Subsequently chemical, electrophysiological, and pharmacological systems are altered, resulting in the maintenance of chronic pain even though the peripheral input is minimal.[26;27] However, besides persistent neural input also other mechanisms may lead to sensitization.[28] Inflammation is a well known contributor[29;30] and it is not unlikely that the pre-existing and/or low-grade residual inflammation of the sarcoidosis may have induced a state of sensitization.

Of note, chronic pain is found to be highly associated with psychological symptoms as depression and anxiety, and with personality disorders like neuroticism.[31;32] A recent study that evaluated personality profiles in chronic pain patients according to Cloninger's model (as we also used in this thesis) revealed that these patients could be characterized by a high Harm Avoidant personality trait.[33] It is remarkable that this same personality profile as well as high levels of depressive and anxious symptoms appeared also to be associated with chronic fatigue in sarcoidosis patients in clinical remission.

Personality

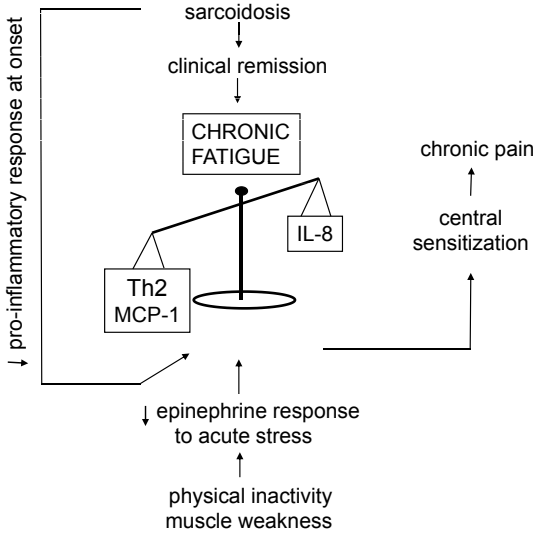
In our search for psychobiological factors that could be associated with chronic post-inflammatory fatigue in sarcoidosis, we observed in chapter 5 a significant association with a complex of a Harm Avoidant personality trait and psychological symptoms, the so-called 'psychoneurotic distress'-cluster. The identification of this complex is in line with other studies that confirm the strong correlation between personality characters and psychological distress[34] In particular, the relation of depression and anxiety disorders with Harm Avoidance has been confirmed in several studies.[35-38]

Literature shows that a neurotic personality profile is significantly associated with quality of life and fatigue at long term follow up in other diseases, like rheumatoid arthritis[39] and cancer.[40;41] Furthermore, personality is generally judged as trait that is heritable and fairly stable over a long time after adulthood.[42-45] In addition, epidemiological studies have shown that individuals who have ever experienced high levels of depression and anxiety, are more likely to report CFS later in life.[46;47] Nevertheless, since our study is cross sectional, we are

unable to address the association of psychoneurotic distress and chronic post-inflammatory fatigue in sarcoidosis in terms of causes and consequences.

Nevertheless, persistence of severe post-inflammatory fatigue may be a crucial factor for the development of clinical depression.[48;49] An important risk factor in this process may be low-grade inflammation.[50-53] To explain the associations between peripheral cytokines and depression various mechanisms have been proposed.[54] One key pathway that may be contributing to the symptoms of depression is activation of the enzyme indoleamine 2,3-dioxygenase (IDO).[55-57] Upon stimulation by cytokines such as IFN-α, IFN-γ, or TNF-α, IDO is produced by macrophages, monocytes, microglia and other immune cells throughout the body. IDO degrades tryptophan into neurotoxic metabolites such as 3-hydroxy-kynurenine and quinolinic acid which easily crosses the blood-brain barrier. Increased activation of IDO leads to the reduced availability of tryptophan and as a consequence the synthesis of serotonin is inhibited.[58] Serotonin plays an important role in the pathophysiology of depression.[59;60] Nevertheless, from animal studies it has become clear that peripheral administration of kynurenine alone can induce depressive-like behavior, indicating that tryptophan depletion may not be required.[61;62] Also in humans, the importance of IDO-activation has been demonstrated. In hepatitis C patients, peripheral administration of IFN-α induced an activation of IDO and cytokine responses, resulting in increased levels of quinolinic acid and kynurenine which correlated significantly with depressive symptoms.[48;57;63] A future study investigating the IDO pathway in our patient group of sarcoidosis patients in clinical remission will be most intriguing.

Figure 1 Hypothetical representation of underlying mechanism of chronic post-inflammatory fatigue in sarcoidosis as examined in this thesis



Follow up

Evaluation of fatigue

Given that all reports in this thesis result from a cross sectional study, we wondered if and how chronic post-inflammatory fatigue in sarcoidosis might change over time.
Therefore, we additionally explored the changes in subjective evaluation of fatigue in our cohort of sarcoidosis patients. All patients who participated in the study, received the fatigue-questionnaire (Checklist Individual Strength (CIS)) and a short checklist to evaluate medical status by regular mail, one year after the day of testing.

Sixty-five out of 75 participants (87% of the total study group) returned the questionnaires: 81% of the patients who had been classified (according the cut-off on the CIS subscale *fatigue-severity*) as fatigued at the first measurement (the 'fatigued group') and 92% of the patients who had previously been classified as non-fatigued (the 'non-fatigued group'). We analyzed the changes in fatigue scores separately for the 'non-fatigued group' and 'fatigued group'. In the non-fatigued group the *fatigue-severity* scores were significantly higher at follow up ($p = 0.029$, see Table 1). Six responders (4 males and 2 females) had a score above the cut-off at follow up.

Table 1 CIS total scores and subscale scores of the **non-fatigued** sarcoidosis patients in clinical remission at the test day and at the follow up after one year. Data are presented as mean ± SEM. If $p < 0.05$, a significant change in means between the two time points was observed.

	Test day	**Follow up**	p value
CIS total score	41 ± 2	46 ± 3	0.159
CIS fatigue-severity	16 ± 1	20 ± 1	**0.029**
CIS concentration	11 ± 0.8	11 ± 1	0.792
CIS motivation	8 ± 0.6	8 ± 0.6	0.663
CIS activity	6 ± 0.4	6 ± 0.6	0.97

In the fatigued group *fatigue-severity* scores were stable over time ($p = 0.652$, Table 2). Only 2 responders had a score below the cut-off at follow up (1 male and one female). Interestingly, subscale scores on *motivation* increased significantly ($p < 0.0001$).
None of the responders reported symptoms that could point to a rechute of sarcoidosis, or reported to be diagnosed by a physician with either reactivation of sarcoidosis, or with any other disease. Nevertheless, the fatigued group indicated to be significantly more anxious that they would face a relapse of the sarcoidosis compared to the non-fatigued group ($p = 0.002$).

Table 2 CIS total scores and subscale scores of the **fatigued** sarcoidosis patients in clinical remission at the test day and at the follow up after one year. Data are presented as mean ± SEM. If $p < 0.05$, a significant change in means between the two time points was observed.

	Test day	**Follow up**	p value
CIS total score	92 ± 2	98 ± 3	0.055
CIS fatigue-severity	45 ± 1	45 ± 1	0.652
CIS concentration	21 ± 1	21 ± 1	0.947
CIS motivation	13 ± 1	18 ± 1	**0.0001**
CIS activity	12 ± 1	13 ± 1	0.265

Predicting total fatigue score at follow up

Now that changes in experienced fatigue over one year had been assessed, we were intrigued by the question which of the psycho-biological factors that were associated with chronic post-inflammatory fatigue in sarcoidosis may predict the observed changes in experienced fatigue at follow up.

We performed a multiple regression analysis and included as predictors for total fatigue score at follow up only those factors that showed significant differences between the fatigued and the non-fatigued participants of our study group. Therefore, the factors 'reduced physical activity' and 'reduced nociception by Aδ fibers' were excluded, because these two factors only showed significant differences when the fatigued subgroup of our cohort was compared to norm scores of a healthy control group.

We ran a multiple linear regression analysis (enter method) including the following factors as predictors for overall fatigue score at follow up:

- subscale Physical functioning of SF-36
  (representing reduced health status, Ch.2)
- percentage of predicted quadriceps muscle force
  (representing reduced muscle strength, Ch.2)
- percentage of days waking up unrefreshed
  (derived from sleep diary, Ch.2)
- Th2 cytokine component
  (derived from the principal component analysis in Ch.3)
- increase in epinephrine concentrations in response to TSST
  (i.e. difference between measurement 'after instruction' and 'after completing the stress tasks', representing changes in neuroendocrine responses to acute stress, Ch.4)
- psychoneurotic distress component
  (derived from the principal component analyses in Ch.5)

- HPA axis component
  (derived from the principal component analysis in Ch.5, representing baseline levels of ACTH/cortisol)
- number of pain points
  (representing elevated levels of daily experienced pain, Ch.6)

The statistical analysis was corrected for the CIS total fatigue score at test day, and for gender.

Multiple regression analysis showed that the model with 8 predictors explained 80.5% of the total variance in the data set. Two out of the 8 factors were identified as significant predictors of the CIS total fatigue score at follow up: the Th2 cytokine component (standardized B = -0.227, p = 0.012) and the psychoneurotic distress component (standardized B = 0.348, p = 0.002, Table 3). Note that the standardized B of the Th2 cytokine component was negative, indicating that decreasing levels of Th2 cytokines predicted increasing levels of total fatigue at follow up.

Table 3 Multiple regression analysis to predict CIS total score at follow up after one year

	Unstandardized Coefficients		**Stand. Coefficients**		**95% Confidence Interval for B**	
	B	**S.E.**	**Beta**	p value	**Lower Bound**	**Upper Bound**
Gender	-2.059	5.96	-0.033	0.732	-14.172	10.055
CIS total score test day	0.630	0.12	0.559	0.001	0.373	0.886
Physical functioning	-0.329	0.21	-0.185	0.140	-0.771	0.144
Quadriceps muscle force	0.089	0.15	0.057	0.572	-0.228	0.407
Days unrefreshing sleep	-0.083	0.12	-0.069	0.502	-0.333	0.167
Th2 cytokines component	-9.158	3.45	**-0.227**	**0.012**	-16.180	-2.136
Epinephrine increase	-4.853	5.48	-0.072	0.383	-6.302	16.007
Psychoneurotic distress component	11.361	3.46	**0.348**	**0.002**	4.324	18.398
HPA axis component	1.402	2.42	0.047	0.566	-3.519	6.322
Number of pain points	0.925	1.32	0.073	0.490	-1.769	3.619

Conclusion on follow up and predictors

Overall experienced fatigue in patients with sarcoidosis after clinical remission of the disease increases over time. At a follow up one year after the test day not only scores on subjective feelings of severe fatigue have been risen, but also subscale scores of motivation have been increased. An attempt to identify psycho-biological factors that could predict total fatigue at follow up resulted in the detection of two factors: the reduced capacity to produce Th2 cytokines in vitro and the constellation of a Harm Avoidant personality in combination with high levels of psychological distress.

Future remarks

Chronic post-inflammatory fatigue in sarcoidosis is a serious problem in which appropriate treatment is of high priority, as it persists over time. The underlying mechanism that maintains post-inflammatory fatigue requires detailed future research. However, from the results of this thesis we propose that the capacity to produce Th2 cytokines may be an important component.

This study has shown that chronic pain is significantly associated with chronic post-inflammatory fatigue in sarcoidosis. Sarcoidosis might have affected the small fibers of all sarcoidosis patients during the active phase of the disease. Subsequently, the damage of these Aδ fibers might persist after resolution of the inflammation in different degrees, resulting in sensitization and chronic pain. In this respect we may classify these chronic pain complaints as neuropathic pain. Over time neuropathic pain may evolve into hyperalgesia or allodynia, which are both severe and resistant to therapy. It is therefore of the utmost importance that sarcoidosis patients are regularly screened for neuropathic pain during the active phase of the disease. Neurological evaluation, specific questionnaires and/or LEP should be part of clinical check-ups of every sarcoidosis patient with active disease.

As described in this thesis, post-inflammatory fatigue in sarcoidosis is significantly associated with a distinct cytokine profile. This profile was assessed by stimulating supernatants of whole blood cultures with either a T cell mitogen (anti-CD2/CD28) or LPS which are both polyclonal stimulators. In future research, we propose to stimulate T cells also by using an antigen that is more specific for sarcoidosis to determine whether the magnitude of the peripheral T cell memory pool, together with the Th1/Th2 cytokine profile, for a given specific disease-associated antigen correlates with fatigue. The so-called Kveim antigen (a preparation of a homogenate of spleen or lymph nodes of sarcoidosis patients that was (up to the 1970s) intracutaneously injected to induce granuloma formation confirming in this way the diagnosis sarcoidosis[64]) might be a good candidate, as long as the exact causative agent for sarcoidosis has not been elucidated yet. In vitro stimulation of T cells with the Kveim-antigen will induce a cytokine/chemokine response of the T memory cells. Cytokine/chemokine production patterns will then show the memory capacity for the former inflammation of sarcoidosis, thereby reflecting the severity of the primary pro-inflammatory immune response. These observations may support our hypothesis that chronically fatigued sarcoidosis patients in clinical remission have had a reduced pro-inflammatory response at the onset of the disease that may have led to a tapered counterbalancing of the immune response afterwards, resulting in chronic fatigue.

Based on this thesis we hypothesize that the reduced capability to produce Th2 cytokines is due to a lower pro-inflammatory immune response at the onset of sarcoidosis, to a non-reactive epinephrine response to stress (in combination with a reduced sensitivity of the immune system to adrenergic stimulation), and/or to a reduction of physical activity levels. However, we acknowledge that also other factors that were not discussed in this thesis may play a role, such as genetic background and epigenetic changes due to severe inflammation. Nevertheless, this thesis has shown that the reduced capability to produce Th2 cytokines is a strong predictor for fatigue severity in the future. Therefore, we propose interventions aiming at restoring the Th2 cytokine production in combination with graded exercise therapy which may lead to resetting of the catecholamine-cytokine balance to the production of more Th2 cytokines.

Furthermore, we found high levels of psychoneurotic distress to be associated with post-inflammatory fatigue in sarcoidosis. Since this theses is a cross sectional study, we were unable to address these symptoms of psychoneurotic distress as either precipitating factors, or the result of the disease, or even the reflection of residual disease. Obviously, longitudinal research in which personality and psychological characteristics are regularly assessed from diagnosis till after resolution of the disease are required.

In addition, follow up of post-inflammatory fatigue in sarcoidosis revealed a significant reduction of motivation over time which is suggestive for the shift from severe fatigue to depression. This move from sickness behavior of which fatigue is a profound feature, to depression has been earlier described in literature.[48;49;57] One of the main factors in this process may be the enzyme indoleamine 2,3 dioxygenase (IDO). In this light, future studies investigating the IDO pathway in our patient group is of incredible interest.
Since we found that a high level of psychoneurotic distress is a strong predictor for severe fatigue at follow up, we propose for the moment that psychological interventions in chronically fatigued patients may profoundly improve their psychological health status.

As this thesis describes post-inflammatory fatigue in sarcoidosis as a serious problem that persists over a long time, immediate treatment of chronically fatigued patients is of the utmost importance. So far, only cognitive behavior therapy and graded exercise therapy have shown to establish significant and stable improvement of fatigue and physical functioning in CFS.[65;66] However, adaptive pacing therapy in which activity levels are carefully matched with the patient's available energy in order to create the best conditions for natural recovery, induced no change in fatigue or physical functioning.[66] These observations are in line with a randomized controlled trial in pain management in which pacing and self-management appeared to be ineffective as well.[67]

Because we do not know whether the underlying mechanism of fatigue in CFS is similar to post-inflammatory fatigue in sarcoidosis, we recommend a trial to assess the effectiveness and safety of cognitive behavior therapy (in order to alleviate psychological symptoms) in combination with graded exercise therapy (so as to enhance physical activity levels and muscle force, but also to stimulate sympathetic output and epinephrine secretion, which subsequently enhances Th2 cytokine production) in a group of chronically fatigued sarcoidosis patients in clinical remission.

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8

Nederlandse samenvatting

Sarcoïdose is een ziekte waarbij ontstekingen ontstaan in verschillende organen van het lichaam. De opeenhoping van witte bloedcellen (granulomen) die daarbij ontstaan, kunnen ertoe leiden dat die organen niet goed werken. Sarcoïdose komt het meest voor in de longen, de lymfeklieren, de huid, de ogen en de gewrichten. Af en toe zijn het hart of het zenuwstelsel aangedaan.

De oorzaak van sarcoïdose is onbekend en er bestaat geen geneesmiddel voor. Naast regelmatige medische controles is meestal geen behandeling nodig; bij de meeste patiënten verdwijnt de sarcoïdose na een paar jaar. Alleen in geval van (kans op) orgaanschade worden ontstekingsremmende middelen voorgeschreven, zoals predniso(lo)n.

Sarcoïdose komt overal ter wereld en bij alle bevolkingsgroepen voor. In Nederland wordt de diagnose bij ongeveer 2000 mensen per jaar gesteld. De ziekte treft evenveel mannen als vrouwen, hoofdzakelijk in de leeftijd tussen de 20 en 40 jaar.

Bij elke patiënt zijn de klachten en de verschijnselen van sarcoïdose weer anders. Deze hangen samen met het orgaan of het weefsel waar de ontstekingen zijn ontstaan. Veel voorkomende klachten zijn: kortademigheid, hoesten, huidproblemen, pijn op de borst, droge/branderige ogen, stijve/pijnlijke enkels en spierpijn. Naast deze problemen die vooral te maken hebben met de plaats van de ontstekingen, zijn er ook meer algemene klachten. Bijvoorbeeld gewichtsverlies, koorts, neerslachtigheid, duizeligheid, hoofdpijn en spierpijn. In dit rijtje hoort ook vermoeidheid thuis. Dit is namelijk één van de belangrijkste klachten van sarcoïdosepatiënten.

Chronische vermoeidheid wordt gedefinieerd als "*het zelf-gerapporteerde lichamelijk en/of mentaal onwelbevinden, langer dan 6 maanden aanhoudend, en zich uitdrukkend als een gevoel van uitputting, als gevolg waarvan iemand lichamelijk en/of mentaal niet kan functioneren op het hem/haar gewenste niveau*". (volgens Vlaams-Nederlands Onderzoeksgroep Chronische Vermoeidheid (VNO-CHROVER))

Chronische vermoeidheid kan niet objectief aangetoond worden middels een laboratoriumtest of meetapparatuur. Het kan alleen in kaart worden gebracht met behulp van een vragenlijst.

Aanleiding en opzet van het onderzoek

Aanleiding

Vermoeidheid is een veel voorkomende klacht bij sarcoïdose. Ook wanneer geen klinische symptomen van de ziekte meer gevonden kunnen worden, blijft vermoeidheid bij veel sarcoïdosepatiënten voortduren. In de literatuur is dit fenomeen beschreven als *post-sarcoïdose chronisch vermoeidheidssyndroom*: een complex van klinische symptomen zoals extreme vermoeidheid, lusteloosheid, diffuse spierpijnen en slaapstoornissen. Vaak zijn de klachten zo ernstig dat deze leiden tot aanzienlijke vermindering van kwaliteit van leven. Ook kan deze vermoeidheid leiden tot arbeidsconflicten in verband met arbeidsongeschiktheid.

Doel

Alhoewel chronische vermoeidheid na sarcoïdose een klinisch en wetenschappelijk erkend probleem is, is er tot op heden geen wetenschappelijk onderzoek naar gedaan. Dit proefschrift bepaalde daarom allereerst de karakteristieken van chronische vermoeidheid wanneer klinische remissie van de sarcoïdose onderkend is. Vervolgens werden psychologische en biologische factoren onderzocht die geassocieerd zouden kunnen zijn met deze chronische post-inflammatoire vermoeidheid bij sarcoïdose patiënten (kortweg *chronische vermoeidheid na sarcoidose* genoemd). We onderzochten vier factoren: cytokine/chemokine profielen; het neuro-endocriene systeem; de nociceptie via perifere zenuwvezels; en persoonlijkheidsfactoren.

Onderzoeksopzet

In dit onderzoek includeerden wij een groep van 75 sarcoïdose patiënten bij wie geen klinische aanwijzingen voor ziekte-activiteit van de sarcoïdose meer detecteerbaar waren. Bij het ontbreken van internationaal geaccepteerde criteria voor ‘actieve ziekte’ en ‘klinische remissie’, werden de criteria voor klinische remissie gebaseerd op bestaande literatuur en klinische expertise. Deze criteria omvatten: lichamelijk, röntgenologisch, longfunctioneel en laboratorium onderzoek. Andere ziekten, aandoeningen en condities die aanleiding zouden kunnen geven tot vermoeidheid, werden uitgesloten. Sarcoïdose moest bij diagnosestelling bewezen zijn door middel van histologie. Bovendien mochten deelnemers minimaal 6 maanden geen corticosteroïden meer hebben gebruikt.

Rekrutering van de patiënten geschiedde middels het aanschrijven van 193 patiënten die waren geselecteerd uit een database van 800 patiënten. Op basis van beschikbare medische gegevens werd ingeschat dat deze patiënten zouden kunnen voldoen aan de in- en exclusie criteria van de studie. Honderdvijftien patiënten reageerden positief op deze uitnodiging, en na telefonisch consult werden 88 patiënten daadwerkelijk gescreend. Vervolgens werden

13 patiënten uitgesloten van deelname vanwege: actieve ziekte (8), Löfgren's syndroom bij diagnosestelling (2), schildklierafwijking (1), diabetes (1), en één patiënt vond deelname te belastend.

Studiepopulatie

In totaal namen 75 sarcoïdose patiënten (in klinische remissie) deel aan de studie. Gemiddelde leeftijd was 47 jaar en de gemiddelde tijd sinds diagnosestelling was 9 jaar. Patiënten met een score van 35 en hoger (de standaard cut-off score) op de subschaal *Ernst-van-vermoeidheid* van de vermoeidheidsvragenlijst Checklist Individual Strength (CIS) werden tot de vermoeide groep gerekend; de overigen tot de niet-vermoeide groep. De niet-vermoeide groep telde 38 patiënten (21 mannen en 17 vrouwen) en de vermoeide groep telde 37 patiënten (12 mannen en 25 vrouwen). Alle analyses werden gecorrigeerd voor deze ongelijke verdeling van mannen en vrouwen.

Er waren geen verschillen in demografische karakteristieken, noch in het gebruik van medicatie tijdens de ziekte of het totaal aantal aangedane organen. Er was wel een verschil in één van de inflammatie-markers op het moment van de diagnosestelling: de niet-vermoeide groep had een significant hoger serum angiotensin converting enzyme (sACE) dan de vermoeide groep.

Chronische post-inflammatoire vermoeidheid na sarcoïdose

Chroniciteit en ernst

Tijdens de actieve fase van de ziekte had 76% van de totale studiegroep klachten van vermoeidheid. Op het moment van de studie had 49% van de studiegroep vermoeidheidsklachten. Kwantificatie met behulp van de CIS liet een gemiddelde score van de totale studiegroep zien van 30.5 op de CISsubschaal *Ernst-van-vermoeidheid*. De niet-vermoeide groep scoorde op deze subschaal gemiddeld 17.16, en de vermoeide groep 44.69. Er waren geen significante verschillen tussen de gemiddelde subscores van vermoeide mannen en vermoeide vrouwen. Omdat de CIS veelvuldig gebruikt is om de ernst van vermoeidheid in kaart te brengen bij andere ziekten en aandoeningen, was een vergelijking daarmee mogelijk. Zo werd duidelijk dat de gemiddelde score van 44.69 relatief hoog was (multiple sclerose: 40.2, functionele buikklachten: 34.1, kankerpatiënten na genezenverklaring: 21.1, alleen in chronisch vermoeidheidssyndroom (CVS) was de score hoger: 51.7).

Karakteristieken

Chronische vermoeidheid na sarcoïdose kenmerkte zich niet alleen door ernstige gevoelens van vermoeidheid, maar ging ook vaak samen met klachten als:

- concentratie- en geheugenproblemen
- pijn (zere keel, spierpijn, hoofdpijn, gewrichten, lymfeklieren)
- malaise en onevenredig lange hersteltijd na inspanning
- niet-uitgerust wakker worden.

Uit de slaapdagboekregistraties bleek dat de vermoeide groep niet langer sliep dan de niet-vermoeide groep, noch 's nachts, noch overdag. Alleen het percentage dagen waarop men onuitgerust wakker werd, bleek hoger te zijn in de vermoeide groep, in vergelijking met de niet-vermoeide groep.

Daarnaast scoorde de vermoeide groep significant hoger op de aanwezigheid van depressieve en angstsymptomen dan de niet-vermoeide groep. Bovendien waren de scores op het gebied van gezondheidsstatus significant lager in de vermoeide groep.

Bij objectieve meting van fysieke activiteit met behulp van een actometer (een soort stappenteller die lichamelijke activiteit en rust registreert) bleken de vermoeide patiënten significant lager te scoren dan de standaard norm. Ook de spierkracht van verschillende spiergroepen (ademhaling, hand, bovenbeen) was in de vermoeide groep significant lager dan in de niet-vermoeide groep.

Psycho-biologische factoren

Naast het vaststellen van de karakteristieken van chronische post-inflammatoire vermoeidheid na sarcoïdose, onderzochten wij de associatie ervan met psychologische en biologische factoren.

Cytokine/chemokine profielen

In hoofdstuk 3 rapporteerden wij de resultaten van de studie naar cytokine/chemokine profielen en de associatie hiervan met chronische vermoeidheid binnen onze studiepopulatie (zie kader voor een korte uitleg over cytokinen en chemokinen). Wij beschreven dat chronische post-inflammatoire vermoeidheid na sarcoïdose is geassocieerd met een specifiek cytokine/chemokine profiel dat zich kenmerkt door een verminderde capaciteit om in vitro Th2 cytokinen te produceren. Daarnaast vonden we in de vermoeide groep gemiddeld meer productie van het chemokine IL-8 na in vitro stimulatie, als ook lagere bloedplasma concentraties van het chemokine MCP-1 in vergelijking met de niet-vermoeide groep.

Tijdens sarcoïdose spelen cytokinen en chemokinen een belangrijke rol. Het begin van de ziekte kenmerkt zich door een surplus aan Th1 cytokinen, terwijl in een later stadium (na granuloomvorming) de balans verschuift naar een overmaat aan Th2 cytokinen. Aangenomen

wordt dat deze omslag van een Th1 naar een Th2 gedomineerde immuunreactie belangrijk is om de immunologische balans te herstellen wat bijdraagt aan herstel van de ziekte.

De bevindingen van onze studie geven aanleiding te veronderstellen dat de immuuncellen van de vermoeide groep minder capaciteit hebben om Th2 cytokinen te produceren waardoor de immuunbalans niet goed hersteld kan worden. Het is onduidelijk wat de oorzaak van deze verminderde capaciteit is. Deze zou gevonden kunnen worden in het neuro-endocriene (hormonaal) systeem. Het is namelijk bekend dat het immuunsysteem en het endocriene systeem wederzijds met elkaar communiceren. Op die manier kan de Th1/Th2 balans ook beïnvloed worden door stresshormonen, met name door cortisol, noradrenaline en adrenaline. Metingen in het bloed lieten zien dat basale cortisol- en noradrenalinespiegels in het bloed niet verschilden tussen de vermoeide en de niet-vermoeide groep. Echter, de adrenalinespiegels van alle patiënten waren in rust hoger dan de normaalwaarden, waarbij de niet-vermoeide mannen de hoogste spiegels vertoonden. Bovendien bleek in reactie op acute stress de verwachte adrenalineafgifte uit te blijven bij de vermoeide patiënten (in de volgende paragraaf wordt deze bevinding verder toegelicht). Dit geeft aanleiding te veronderstellen dat veranderingen in de respons van adrenaline een rol zouden kunnen spelen in de verminderde capaciteit Th2 cytokinen te produceren.

Cytokinen en chemokinen

Wanneer het immuunsysteem in actie komt, produceert het eiwitten. Deze eiwitten functioneren als boodschapperstoffen die de communicatie mogelijk maken tussen verschillende immuuncellen, maar ook tussen het immuunsysteem en andere organen (zoals de hersenen en het zenuwstelsel). Deze eiwitten heten cytokinen en chemokinen. Chemokinen bezitten de specifieke eigenschap om leukocyten (witte bloedcellen) aan te trekken naar de ontstekingshaard, een proces dat ook wel ‘chemotaxis’ wordt genoemd. Cytokinen worden door veel cellen in het lichaam gemaakt. Wanneer ze door T cellen gemaakt worden, kunnen we cytokinen indelen en benoemen analoog aan het type T cel: de T helper 1 (Th1) cytokinen en de Th2 cytokinen.

Naast het reguleren van het immuunsysteem, kunnen cytokinen en chemokinen ook een rol spelen in het veroorzaken van niet-specifieke ziektesymptomen tijdens ziekte: vermoeidheid, malaise, neerslachtigheid en pijn. Dit fenomeen wordt ook wel *ziekte-gedrag* of *sickness behavior* genoemd.

In dit onderzoek hebben we cytokinen en chemokinen gemeten in het bloedplasma van sarcoïdosepatiënten in klinische remissie. Daarnaast hebben we de natuurlijke immuunreactie in het laboratorium (in vitro stimulatie) nagebootst. Zo kregen we een indruk van het functioneren van hun immuunsysteem.

Neuro-endocrien systeem

Hoofdstuk 4 onderzocht het neuro-endocriene systeem als mogelijke factor die verband zou kunnen houden met de aanwezigheid van chronische vermoeidheid na sarcoïdose. Het immuunsysteem staat namelijk in wederzijdse verbinding met het neuro-endocriene systeem. In deze studie waren wij vooral geïnteresseerd in het vermogen van het neuro-endocriene systeem om te reageren op acute stress. Wij testten dit door middel van de Trier Social Stress Test, een test waarbij de deelnemer onvoorbereid wordt blootgesteld aan een spreekopdracht (simulatie sollicitatie) en een hoofdrekentaak die beide uitgevoerd moeten worden ten overstaan van publiek en/of een videocamera. Het is gebleken dat deze test stressreacties uitlokt die onder andere kunnen worden geobserveerd als veranderingen in hartslag en bloeddruk, en in het bloed gemeten kunnen worden in stresshormonen, zoals ACTH, cortisol en (nor)adrenaline.

In ons studiecohort zagen wij geen verschillen tussen vermoeide en niet-vermoeide patiënten in reactie op acute stress wat betreft de volgende stresshormonen: cortisol, ACTH en noradrenaline. Ook qua reactie in bloeddruk waren er geen verschillen. Echter, vermoeide vrouwen hadden gedurende de gehele test een lagere bloeddruk in vergelijking met de niet-vermoeide vrouwen. Daarnaast bleef de verwachte toename in adrenaline uit bij zowel vermoeide mannen als vermoeide vrouwen. Normaliter piekt de adrenaline concentratie na het afronden van de twee stresstaken, maar bij de vermoeide patiënten bleek de hoogste concentratie al juist daarvoor bereikt te zijn. Het uitblijven van de verwachte adrenaline respons in de vermoeide groep was opmerkelijk, zeker in het licht van de verhoogde basale adrenaline spiegels. Men zou kunnen veronderstellen dat de vermoeide groep niet in staat was om bovenop deze verhoogde spiegel adequaat te reageren op acute stress omdat de maximum concentratie reeds bereikt is. Verder zou een oorzaak gezocht kunnen worden in de verminderde fysieke activiteit en spierzwakte van de vermoeide groep. Het is namelijk bekend dat fysieke inspanning het sympathisch zenuwstelsel activeert en bijdraagt aan een gezonde adrenaline respons.

Het effect van een bepaald hormoon hangt echter niet alleen af van de (wisselende) concentraties in het bloed, maar ook van de gevoeligheid van de receptoren op de immuuncellen voor dat hormoon. In deze studie testten we daarom met behulp van een laboratoriumtest ook deze receptorgevoeligheid van de immuuncellen in het bloed voor cortisol (glucocorticoids) en adrenaline (terbutaline). Hieruit bleek dat de immuuncellen van vermoeide vrouwen minder gevoelig waren voor adrenaline dan die van niet-vermoeide vrouwen. Bij mannen werd hierin geen verschil gevonden tussen de vermoeide en de niet-vermoeide groep. Ten aanzien van de receptorgevoeligheid voor cortisol waren er geen verschillen tussen de vermoeide en de niet-vermoeide groepen.

Samenvattend ontstaat het beeld dat vermoeide sarcoïdosepatiënten in klinische remissie een verminderde adrenaline respons hebben wanneer zij worden blootgesteld aan acute stress. Daarbij komt dat de immuuncellen van vermoeide vrouwen ook nog eens minder gevoelig lijken te zijn voor adrenaline. Hoewel de oorzaak van deze ontregelingen niet bekend is, vermoeden wij dat fysieke inactiviteit en spierzwakte een rol zouden kunnen spelen.

Nociceptie via perifere zenuwvezels

Na het immuunsysteem en het neuro-endocriene systeem, belichtte hoofdstuk 5 het nociceptieve systeem (pijnsignaleringssysteem). Chronische vermoeidheid en pijn gaan immers vaak samen. Bovendien is vastgesteld dat sarcoïdosepatiënten met actieve ziekte kunnen lijden aan vermindering van het aantal uiteinden van de dunne vezels in de huid, hetgeen neuropathische pijn tot gevolg kan hebben. In deze studie onderzochten wij de nociceptie via perifere zenuwvezels met behulp van een lasertechniek (laser evoked potentials – LEP) in combinatie met registratie van elektrische impulsen in de hersenen (electro encephalo gram – EEG). De patiënten kregen laserstralen in verschillende sterktes op hun voet. Dunne vezels, ook wel Aδ vezels, geleidden deze pijnimpulsen via het ruggenmerg en de laterale spinothalame baan naar de somatosensorische hersenschors. Registratie aldaar kan zichtbaar gemaakt worden met behulp van het EEG.

Uit de studie bleek dat sarcoïdosepatiënten in klinische remissie pijnprikkels van de hoogste lasersterkte (2.0 Watt) wel net zo snel, maar minder goed registreerden in vergelijking met een gezonde controlegroep. De verschillen zijn echter erg klein, hangen niet samen met vermoeidheid, en we weten ook niet waar in het systeem dit defect precies optreedt. Omdat aangetoond is dat huidbiopten van patiënten met actieve sarcoïdose minder vezeluiteinden bevatten, vermoeden wij dat de schade zich aldaar bevindt. Aangezien schade aan zenuwvezels kan leiden tot neuropathische pijn, vinden wij hierin ook een mogelijke oorzaak voor de verhoogde dagelijkse pijnklachten van de vermoeide patiënten. Een andere verklaring zou kunnen zijn dat er bij deze patiënten sensitisatie (overgevoeligheid) is opgetreden. Dit kan gebeurd zijn ten gevolge van overmatige prikkeling van beschadigde zenuwvezels, maar ook door processen van immunologisch aard, namelijk de hevige immunologische respons van het ziekteproces (de sarcoïdose) zelf of het langdurig blootgesteld zijn aan laag-gradige immuunactiviteit.

Persoonlijkheid

Vervolgens testte hoofdstuk 6 of persoonlijkheidkenmerken al dan niet in combinatie met psychologische symptomen en stresshormonen gecorreleerd zijn met chronische vermoeidheid na sarcoïdose.

Uit de analyse bleek dat in onze patiëntengroep psychologische symptomen (angst en depressie) in hoge mate samenhingen met een persoonlijkheid die zich kenmerkt door leedvermijdend gedrag. Deze constellatie van symptomen (in hoofdstuk 6 ook wel de *psychoneurotische component* genoemd) bleek significant geassocieerd met vermoeidheid. Ook de basale spiegel van stresshormonen (ACTH en cortisol) in het bloed bleek een voorspeller van vermoeidheid te zijn, waarbij verlaagde hormoonspiegels samenhingen met vermoeidheid. Opgemerkt moet worden dat het onduidelijk is of de psychoneurotische symptomen reeds voor de diagnose bestonden, dan wel tijdens het ziekteproces of nadien ontstaan zijn, omdat informatie op deze tijdsmomenten niet voor handen is. Overigens wordt algemeen aangenomen dat persoonlijkheidskenmerken als een leedvermijdend temperament aangeboren zijn en in volwassenheid nauwelijks meer veranderen.

Follow up

Om een indruk te krijgen van het verloop van de vermoeidheidsklachten ontvingen alle patiënten één jaar na de testdag per post de vermoeidheidsvragenlijst met het verzoek deze opnieuw in te vullen. Vijfenzestig van de 75 patiënten (87% van de totale studiegroep) retourneerden de vragenlijst. De groep patiënten die tijdens de testdag waren geclassificeerd als 'niet-vermoeid' scoorden bij de follow up significant hoger op de subschaal *Ernst-van-vermoeidheid*: 6 patiënten scoorden bij follow up zelfs hoger dan de standaard cut-off score voor vermoeidheid. Deze gegevens wezen op een toename in vermoeidheid.

Echter, in de groep die op de testdag als vermoeid werd geclassificeerd waren de vermoeidheidscores gelijk gebleven; alleen de subschaal *Reductie-van-motivatie* liet significant hogere scores zien. Geen enkele respondent gaf aan een opleving van de sarcoïdose te hebben gehad of te zijn gediagnosticeerd met een andere ziekte.

Uit een aanvullende analyse bleek dat de mate van vermoeidheid bij follow up met name voorspeld kon worden door de verminderde capaciteit Th2 cytokinen te produceren en door de psychoneurotische component.

Conclusie en aanbevelingen

Chronische vermoeidheid na sarcoïdose is een important probleem waarbij directe en adequate behandeling geïndiceerd is, omdat het probleem toeneemt in de loop van de tijd. Naar de oorzaak ervan moet nog meer onderzoek gedaan worden. In dit proefschrift werd aangetoond dat chronische vermoeidheid bij sarcoïdose patiënten in klinische remissie samenhangt met een veranderd cytokine/chemokine profiel, een inadequate adrenaline respons, spierzwakte, pijn en psychoneurotische symptomen. Onlangs is wetenschappelijk bewezen dat cognitieve gedragstherapie en graduele oefentherapie (hierbij verhogen patiënten hun fysieke inspanningen volgens een schema, ongeacht hoe ze zich voelen) de vermoeidheid bij patiënten met CVS langdurig verminderen. Hoewel het onderliggend mechanisme van chronische vermoeidheid na sarcoïdose mogelijk verschilt van dat bij CVS, steunen de bevindingen in dit proefschrift een prospectieve studie naar de effectiviteit van cognitieve gedragstherapie (ter verlichting van de psychoneurotische symptomen) en begeleide fysiotherapie (niet alleen om de fysieke conditie en spierkracht te verbeteren, maar ook om de adrenaline respons te stimuleren die vervolgens zou kunnen leiden tot een verbeterd immunologisch evenwicht).

List of abbreviations

ACE	angiotensin converting enzyme
AUC	area under the curve
BMI	body mass index
CFS	chronic fatigue syndrome
CIS	checklist individual strength
CRP	c-reactive protein
EDTA	ethylene diamine tetra acetic acid
HPA axis	hypothalamus pituitary adrenal axis
IFN-γ	interferon-γ
IL	interleukin
IQR	interquartile range
IL-Ra	interleukin receptor antagonist
IP-10	interferon-gamma induced protein-10
LEP	laser evoked potentials
LPS	lipopolysaccharide
MIP	macrophage inflammatory protein
MCP-1	monocyte chemoattractant protein-1 (also CCL2)
RANTES	regulated on activation, normal T expressed and secreted (also CCL5)
sIL-2R	soluble interleukin-2 receptor
SF-36	medical outcomes study 36-item short-form health survey
SNS	sympathetic nervous system
Th cell	T helper cell
TNF-α	tumor necrosis factor-α

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Epiloog

Dit proefschrift bevat een kleine bijdrage aan de wetenschap. Een kleine. Want hetgeen dit proefschrift aan wetenschappelijk resultaat oplevert, kan ik wellicht samenvatten in drie zinnen (of stellingen). Wat er aan vooraf ging, strekt zich uit over jaren. Wetenschappelijk gezien bijna vijf. Maar met iets meer helikopterview is het misschien wel het resultaat van de loop van het leven. Zo leerde mijn moeder mij als kind succesvol plannen en organiseren; bracht mijn vader me bij alle zintuigen te gebruiken (lang voordat mindfulness een hype werd); samen gaven ze mij de ruimte te experimenteren, te creëren en te fantaseren. Het plezier in observeren en het-zich-afvragen erfde ik wellicht van mijn opa. Aan deze prachtige basis voegde zich een paar jaar geleden de aanstekelijke bevlogenheid van mijn echtgenoot voor de wetenschap: zie hier de ingrediënten voor een proefschrift.

Anderzijds kan dit boekwerkje gezien worden als het resultaat van vertrouwen. Aan het begin van de vijf wetenschappelijke jaren speelden in dat opzicht een belangrijke rol: Heleen van Velzen-Blad, Paul Kok, Cobi Heijnen en vooral Jules van den Bosch die onlangs op 1 december 2010 overleed. Het is zo verdrietig te ervaren dat diegene die ik veel dank verschuldigd ben, nu niet meer bij ons is…

Ook weerspiegelt dit proefschrift de vrucht van samenwerking. Tijdens de opstartfase had ik het geluk bijgestaan te worden door deskundigen die bereid waren hun kennis met mij te delen, waaronder Maike ter Wolbeek, Paula Mommersteeg, Hanneke Dolk en Marijke Tersteeg-Kamperman.

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Wetenschappelijk gezien kan dit proefschrift misschien samengevat worden in een drietal stellingen, qua proces in drie woorden: samenwerking, vertrouwen en levensgeluk.

En nu?
Nu dit is volbracht?
“Gewoon doorgaan. En… volhouden!”
Ik knipoog stilletjes naar boven…

Curriculum vitae

Ingrid Hermanna Elisabeth Korenromp was born on April 25 1970 in Beltrum (Eibergen), the Netherlands. After graduating from the RK Scholengemeenschap Marianum in Groenlo in 1988, she studied social behavioral sciences at the St. Radboud University of Nijmegen and specialized in behavior disorders in young children. Meanwhile, she was trained as a primary school teacher at the PABO Groenewoud in Nijmegen and next at the Hogeschool Midden Nederland in Utrecht. She married in 1993, and 3 years later she had given birth to 4 children. During a sabbatical leave with her family in 2001 (Abingdon, Oxford, UK), she studied English language, while working as a freelance writer and till 2005 also as the editor of a journal on ceramics. In that same year she was caught by the 'research-virus', starting as an employee of the Lung Research Database of the Heart Lung Center Utrecht (HLCU). In 2006 she designed her PhD-project on chronic post-inflammatory fatigue in sarcoidosis, a close collaboration of the Center of Interstitial Lung Diseases of the department of Pulmonology, St. Antonius Hospital Nieuwegein (supervisors: prof.dr. J.M.M. van den Bosch, succeeded by prof.dr. J.C. Grutters), and the University Medical Center Utrecht, Laboratory of Neuroimmunology and Developmental Origins of Disease (supervisors: prof.dr. C.J. Heijnen and dr. A. Kavelaars). During this period she was trained in Good Clinical Practice in medical research involving humans subjects, and in multilevel statistics, she supervised the dissertations of two nurse-practitioners, and wrote a research protocol on the influence of successive cardiopulmonary exercise testing on fatigue in active sarcoidosis that was granted funding by the Dutch Sarcoidosis Society (SBN).

Vermoeidheid komt voor bij veel ziekten.
Ook bij sarcoïdose. Maar veel sarcoïdose patiënten houden ook nog klachten als de arts geen aanwijzingen meer voor ziekte kan vinden.
Chronische vermoeidheid is dan de hoofdklacht.
Hierover gaat dit proefschrift.
Het beschrijft vermoeidheid na sarcoïdose,
en klachten en symptomen waarmee het samengaat.
Daarnaast zoekt het naar factoren die verband kunnen houden met vermoeidheid na sarcoïdose.
Het proefschrift belicht vier factoren:
het afweersysteem, het hormonaal stelsel, de pijngeleiding via dunne vezels en tenslotte de persoonlijkheid.