

Chapter 1

General introduction



Introduction

It is well established that sarcoidosis is a multisystem disorder of unknown cause(s). To date, no organ is immune to sarcoidosis. Granulomas, usually noncaseating but occasionally necrotizing, are the pathological hallmark of sarcoidosis. Granulomas are structured masses composed of activated macrophages and their derivatives, *i.e.* epithelioid and giant cells^{1,2}. Granulomas are also features of many other (interstitial lung) disorders, *e.g.* extrinsic allergic alveolitis or hypersensitivity pneumonitis, drug-induced pneumonitis, chronic berylliosis, and Crohn's disease. Sarcoidosis subsides in most cases, but it may worsen and become chronic in others. Pulmonary problems may persist, but also devastating extrapulmonary complications may become apparent. Appropriate management of sarcoidosis is mandatory as it predominantly affects fairly young adults (between 20 - 40 years). To date, it requires the attention of pulmonologists as well as specialists from other medical disciplines. Therefore, a multidisciplinary approach is recommended that pays attention to the many aspects of this erratic disorder³.

Sarcoidosis

Etiology and epidemiology

Sarcoidosis is a disseminated, granulomatous disease of unknown etiology that occurs throughout the world. To date, it is the second most common respiratory disease in young adults after asthma. Estimates of prevalence range from 1 to 50 per 100.000 individuals, varying among ethnic and racial groups⁴. The disease is most common among North Americans of African heritage and Northern European Caucasians (The Netherlands and Germany: 40 – 50 cases per 100.000). Various infectious, organic, and inorganic agents are considered to be responsible for a granulomatous reaction in susceptible hosts⁴⁻⁷. It is highly likely that a genetic susceptibility to the development of sarcoidosis exists⁸⁻¹¹.

Clinical presentation

The clinical course of sarcoidosis is highly variable and unpredictable. Virtually every organ can be involved. The lungs are affected in over 90% of patients with sarcoidosis⁴. It also frequently affects the lymph nodes, skin, and eyes.

The acute form is known as Löfgren's syndrome, defined by an acute onset of symptoms of fever, erythema nodosum, hilar lymph adenopathy with or without arthralgia. To date, sarcoidosis patients not only suffer from symptoms related to the lungs (*e.g.* cough, breathlessness), but they may also suffer from a wide spectrum of other symptoms^{4,12}. These symptoms include persistent fatigue, arthralgias, muscle pain, weight loss, skin lesions, eye problems, and neurological as well as cardiological problems^{4,12,13}. These rather non-specific symptoms are disabling for the patient, may become chronic and causing an impaired quality of life¹³⁻¹⁹. Fatigue, as an integral part

of the clinical picture of sarcoidosis, has gained some attention in the literature^{12,20-24}, although the exact mechanism is still unknown.

Generally, spontaneous remission occurs in 75 – 85% of the cases within two years. However, the disease can also become chronic and progressive, estimated in up to 15 – 25% of the cases. Failure to regress spontaneously within two years is considered to predict a chronic course of the disease. Overall, the mortality from sarcoidosis is 1 – 5%. Treatment of sarcoidosis consists largely of immunosuppressive drugs such as steroids, methotrexate, cyclosporin and, more recently, anti-TNF α (Infliximab)^{25,26}.

Diagnostic work-up and follow-up

A pulmonary physician generally coordinates management of sarcoidosis. However, sarcoidosis can involve any organ. The diagnostic work-up and follow-up of the disease is mainly monitored by assessing clinical features and using auxiliary diagnostic procedures. According to the latest international recommendations, the diagnostic work-up includes attention to the medical history, relevant exposures, imaging procedures, laboratory tests, bronchoalveolar lavage²⁷ and, if appropriate, evaluation of biopsy specimens⁴. According to the latest recommendations as published by Baughman *et al.*, diagnosis of sarcoidosis can be established by means of the following criteria:²⁸

- The presence of granulomas in biopsy specimen without evidence of tuberculosis, fungus, malignancy or other causes of granuloma together with clinical features of sarcoidosis support the diagnosis.
- In the absence of biopsy material, clinical features such as bilateral hilar adenopathy on chest radiography, erythema nodosum, uveitis and macular papular skin lesions are suggestive of sarcoidosis.

If there is no evidence of an alternative diagnosis and additional features highly consistent with sarcoidosis are present such as raised angiotensin-converting enzyme (ACE), bronchoalveolar lavage (BAL) fluid lymphocytosis and/or lupus pernio (cutaneous manifestation of sarcoidosis frequently on the nose), the diagnosis can also be confirmed^{4,28}.

The recommended clinical tests in the management of sarcoidosis are presented in Table 1.1. Among others, lung function test results are used to guide the therapeutic approach. Depending on the severity of the inflammation in the lung, lung function tests may reveal restricted lung volumes and/or impaired diffusion capacity and mildly decreased arterial oxygen pressure at exercise. Pulmonary and mediastinal lymph node involvement is found in up to 90% of patients, which is easily recognizable on chest radiography²⁹. Intrathoracic involvement can vary from sole bilateral hilar lymphadenopathy to extensive parenchymal disease and fibrosis. In general, chest radiographic appearances have been divided into four ‘stages’ or types according to the modified Scadding criteria: stage 0, no lung involvement; stage I, bilateral hilar enlargement alone; stage II, hilar enlargement in association with interstitial lung disease; stage III interstitial lung disease alone; and stage IV (a more recent addition to the original classification), requires radiographic evidence of lung fibrosis³⁰.

Table 1.1. Recommended initial evaluation of patients presenting with sarcoidosis^{4*}.

1.	History	Exposure: - occupational - environmental Symptoms
2.	Physical examination	
3.	Chest radiography (postero-anterior)	
4.	Pulmonary function tests	Spirometry DLCO
5.	Peripheral blood counts	White blood cells Red blood cells Platelets
6.	Serum chemistries	Calcium Liver enzymes: - alanine aminotransferase - aspartate aminotransferase - alkaline phosphatase Creatinine Blood urea nitrogen
7.	Urine analysis	
8.	ECG	
9.	Routine ophthalmologic examination	
10.	Tuberculin skin test	

* In the initial evaluation of sarcoidosis no ACE measurement was recommended.

Disease activity and severity

In general the state of a disease can be classified either by activity and/or severity. Unlike other disorders, activity in sarcoidosis does not necessarily indicate a progressive course, a fatal prognosis, or the need for medical treatment³¹.

Moreover, there is a lot of discussion about the definitions of both, as it is very difficult to make a clear distinction between these two entities. Besides, there is overlap between the two. The activity is defined in the latest World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) statement on sarcoidosis (1999). According to the statement, clinical activity is assessed on the basis of onset, worsening, or persistence of symptoms or signs directly related to sarcoidosis. The statement, however, does not give a clear recommendation on how to define the activity (Table 1.2.)⁴. Unfortunately, the term “activity” has in the past been used freely and not always systematically³².

Inconsistency exists also on how to define severity. Changes in plain chest radiography and lung function tests have traditionally been used as the main indices of change of respiratory function indicating pulmonary severity³². Extrapulmonary manifestations such as cardiac, neurological or ocular lesions often also represent a more severe course of the disease. Many new parameters are evaluated as activity parameters, whereas the concept of severity is left out. Recently, some studies evaluated new parameters with the background of severity³³⁻³⁵. Moreover, among others one genetic study appeared which showed that human leukocyte antigen (HLA)-DQB1 allele *0201 is strongly protective against severe sarcoidosis⁸.

Until now, far less attention has been paid to severity of the disease and the appropriateness of the various markers to detect severity. From a clinical point of view, it is of more importance to depict whether the disease is severe, *i.e.* will have a more or less chronic course than the initial activity. For each appropriate evaluation of the diagnostic accuracy of any marker a clear definition of the condition such as activity or severity is needed. However, a problem arises in case of sarcoidosis.

Therefore, one of the aims of this thesis was to search for new markers, which could possibly reflect disease severity in sarcoidosis. In case of pulmonary sarcoidosis disease severity was defined through the presence of respiratory functional impairment.

The ideal marker(s) of disease severity should be able to predict subsequent organ dysfunction. However, such markers are not identified by now³².

Table 1.2. Markers of disease activity in sarcoidosis⁴.

Clinical	Biochemical or instrumental	Imaging
Fever	Serum ACE	Progressive changes on chest radiographs or lung CT scans
Eye involvement uveitis	Hypercalcemia	Ground-glass attenuation on HRCT scan
Skin involvement - Erythema nodosum - Lupus pernio - Changing scar	Worsening lung function	Positive 67Ga uptake (panda/labda sign)
Joint involvement - Polyarthralgia	BAL fluid: lymphocyte alveolitis and high CD4/CD8 ratio	Fluorescein angiography of the eyes
Splenomegaly	Abnormal ECG, echocardiogram or thallium scan	MRI or CT scan abnormalities
Enlarged lymph nodes (lymphadenopathy)		
Salivary and lacrimal gland enlargement	Abnormal liver function tests	Bone cysts
Myocardial disease		
Unilateral facial paralysis or other neurological symptoms/signs		
Progressive respiratory symptoms (dyspnea, cough)		

Molecular changes in sarcoidosis

Inflammation that does not resolve within a matter of days (by means of “natural immunity”), becomes chronic, as is the case in sarcoidosis. Antigens are than usually presented to the lymphocytes by antigen presenting cells (APC). These APC’s (dendritic cells, macrophages and sometimes B cells) trap antigens, concentrate them, prevent their spread and initiate local immune response.

Before an antigen can be presented to a T cell, it must be processed by an APC. This involves the enzymatic degradation of the antigen and anchoring to highly polymorphic class I (variants of HLA -A, -B, -C) or class II (variants of HLA -DP, -DQ and -DR) major histocompatibility complex (MHC) molecules³⁶⁻³⁹. In sarcoidosis associations with the different HLA molecules have been observed, mainly with the subtypes of HLA-B, HLA-DR and HLA-DQ⁸⁻¹⁰. MHC class I interact with CD8⁺ T cells, whereas MHC class II interact with CD4⁺ T cells (see further)³⁶⁻³⁹.

After the presentation of antigen by the APC and reaction with a processed antigen, T cells go through stages of activation, growth and differentiation and secrete a variety of mediators (cytokines). These cytokines are especially important in regulating the function of other lymphocytes. T cells express particular accessory molecules on their cell surface that are important to their function but can also be used to classify them. The T cells expressing CD4 molecules (*i.e.* T-helper cells) are involved in either cell-mediated immunity (Th1 cells, also called delayed hypersensitivity T cells) or promote the activation, growth and differentiation of B cells (Th2 cells). In contrast T cells expressing CD8 molecules, either counterbalance the effects of T-helper cells and suppress immune response (T-suppressor cells) or are involved in the killing of other cells (cytotoxic-T cells). The measurement of T cells expressing either CD4 or CD8 has become clinically important as changes in their balance (CD4/CD8 ratio) may be an indication of a disease³⁶⁻³⁹.

In the sarcoid lung, the earliest pathologic finding is a mononuclear alveolitis composed of increased CD4⁺ T-helper lymphocytes (with an increased CD4/CD8 helper/suppressor ratio), monocyte-macrophages and some B-lymphocytes^{4,40}.

Also in sarcoidosis, the consequence of antigen presentation to specific T-lymphocytes is amplification of the immune response through proliferation of T-lymphocytes and their production of cytokines^{4,28,41}. The APC-lymphocyte conjugation transduces a signal to the lymphocyte to express IL-2 receptors on T-lymphocyte cell surfaces and release IL-2 (Figure 1.1). The binding of IL-2 to IL-2 receptors causes proliferation of IL-2-responsive lymphocytes. The proliferating T-helper cells command the assembly of the granuloma through production of various molecules^{4,28,41,42}. In fact, in the early stage of the disease, the local tissue inflammation in sarcoidosis is dominated by expression of Th1-cytokines (mainly IL-2, interferon- γ (IFN- γ) and tumor necrosis factor- β (TNF- β))⁴³⁻⁴⁹.

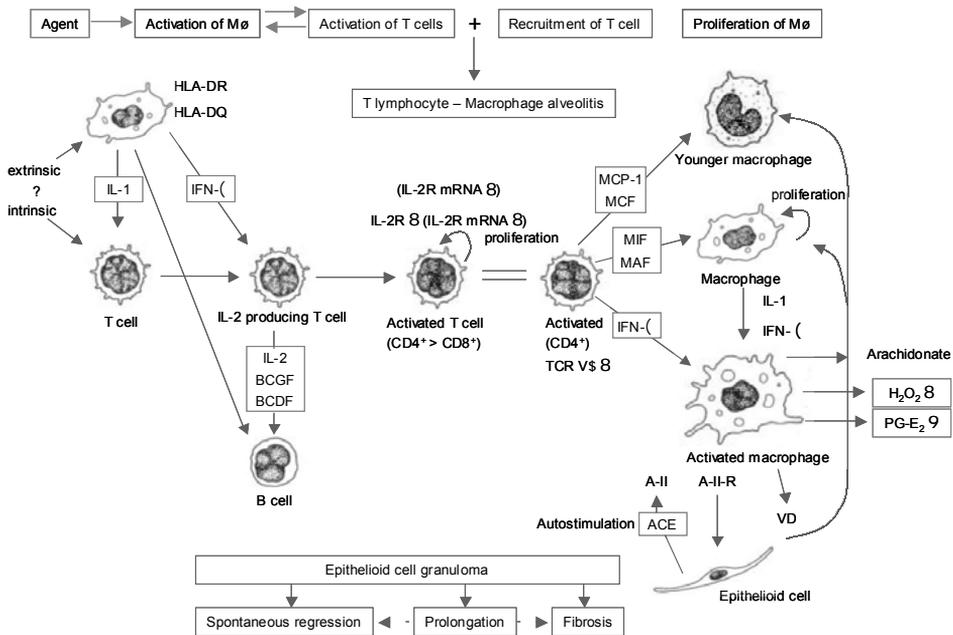


Figure 1.1. Pathophysiological processes in patients with pulmonary sarcoidosis (adapted with permission from Nagai *et al.*⁴¹).
 Mφ = macrophages; IL-1 = interleukin-1; IL-2R = IL-2 receptor; BCGF = B cell growth factor; BCDF = B cell differentiation factor; MCP-1 = monocyte chemotactic peptide-1; MCF = monocyte chemotactic factor; MIF = macrophage inhibition factor; MAF = macrophage activating factor; IFN- γ = interferon γ ; PG-E2 = prostaglandin E2; A-II = angiotensin II; A-II-R = angiotensin II receptor; VD = activated vitamin D (1 α , 25-dihydroxy-form); TCR $\nu\beta$ = T cell receptor $\nu\beta$.

Sarcoid lung T-lymphocytes spontaneously release several cytokines such as soluble monocyte chemoattractant factor (MCF) to attract the monocytes. Monocytes are the predominant cellular resource required for granuloma architecture. Recruited monocytes become activated and sequentially differentiate into macrophages, epithelioid cells, and giant cells. These granuloma structures (mainly epithelioid cells) are able to produce angiotensin-converting enzyme and the active form of vitamin D as shown in Figure 1.1. An increased production of oxygen peroxide (indicating presence of oxidative stress) and prostaglandin E2 has also been observed (Figure 1.1)^{41,50}.

The net effect of the Th1 (IFN- γ dominant) response is the organization of the local inflammatory process into the granuloma and an inhibition of fibrogenetic processes⁴⁶. In addition to the T cell activation also some B cell activation occurs which may result in hypergammaglobulinemia⁵¹.

Nevertheless depending on the host susceptibility, a switch to type 2 (IL-4-dominant) may occur in patients with progressive sarcoidosis who evolve towards lung fibrosis^{46,52}. The identity of molecules and receptors that drive the Th1/Th2 balance at sites of disease activity remains controversial⁴⁶.

Outline of the serum markers evaluated in this thesis

Acute phase response

As a consequence of the inflammatory stimuli, IL-1 and IL-6 are produced, which stimulate liver production of C-reactive protein (CRP) and serum amyloid A (SAA), among other proteins.

C-reactive protein

C-reactive protein (CRP) is an acute-phase protein featuring a highly conserved structure⁵³. Expression of CRP is regulated mainly at the transcriptional level with IL-6 being the principal inducer of the gene during the acute phase. It is built up of five identical subunits, aggregated in a symmetric pentameric form by noncovalent binding between the subunits. Each subunit has the ability to bind two calcium ions so that a calcium-dependent specific binding of a ligand is possible. The most avid ligand is phosphocholine (PCh). The PCh is widely distributed in pathogens and in cellular membranes^{53,54}.

CRP is involved in opsonization and phagocytosis through two routes: activation of the classical pathway of the complement and through the direct binding to the Fc fragment of the IgG receptors. Interestingly, expression of Fc receptors was shown to be suppressed in alveolar macrophages from patients with sarcoidosis⁵⁵.

Clearance of CRP is monoexponential and independent of the serum concentration or pathophysiological circumstances, what normally makes the CRP a good marker of disease activity.

The measurement of plasma or serum CRP can help differentiate inflammatory from non-inflammatory conditions⁵³. In rheumatoid arthritis and pancreatitis CRP measurement has prognostic value^{53,56}. Since inflammation is believed to play a role in the pathogenesis of cardiovascular events, measurement of CRP has been proposed for the prediction of the risk of these events^{57,58}. Also in sarcoidosis CRP appeared to be increased especially in acute disease³⁴.

Serum amyloid A

Serum amyloid A (SAA) is a polymorphic protein that consists of four genetically determined isotypes. Isotypes SAA1 and SAA2 are synthesized in response to inflammatory cytokines and present together the SAA response measured as a part of the acute phase reaction^{59,60}. The gene encoding SAA3 is not expressed, whereas SAA4 present a constitutive apolipoprotein in HDL, and it does not behave as acute phase reactant.

SAA (SAA1 and SAA2) synthesis is induced primarily in the liver, by the stimulation of inflammation-related cytokines such as IL-1, IL-6 and TNF- α ^{61,62}. However, it appears that there is also some extrahepatic SAA synthesis. The plasma concentration of SAA changes can be very large due to inflammatory stimuli (1000x increase). The major role of SAA appears to be preserving homeostasis by preventing further progress of injuries or by repairing damaged tissues. This mainly because some of the following

functions have been attributed to SAA: 1. inhibition of antibody production by lymphocytes, 2. inhibition of platelet agglutination; 3. inhibition of the oxidative burst reaction in neutrophils; 4. induction of collagenase and 5. chemotaxis of neutrophils and monocytes^{59,60}.

Immediately after SAA is released from the cells, it binds with high-density lipoproteins (HDL) and circulates throughout the system. The role of HDL is to remove cholesterol from the tissues. Circulating HDL acquires cholesterol by extracting it from cell surface membranes and converts it into cholesterol esters through the action of lecithin cholesterol acyltransferase (LCAT), an enzyme that is activated by the HDL component apoA-I. In the circulation, SAA is rapidly cleared. Inflammatory conditions that are accompanied with high SAA concentrations greatly alter the HDL composition, reducing levels of apoAI, and increasing those of SAA. Reduction of apoAI is due to lowered synthesis in the liver and probably displacement from HDL by SAA^{59,60}, which has been shown in sarcoidosis, too⁶³.

In drawn plasma, almost all SAA is present in high molecular form (most in HDL and little in other lipoproteins). Most SAA assays are directed to SAA in HDL, and not to SAA monomer, which reacts with antibodies differently from the lipid-bound form. Furthermore, for measurement of acute phase SAA, cross-reactivity of antibodies with SAA4 must be avoided.

Clinical value of the SAA has been established in various diseases. During viral infections, SAA appeared to increase more intense as compared to CRP^{59,64,65}. In some diseases, such as Crohn's disease and ulcerative colitis, CRP remained even normal, whereas SAA increased⁶⁶⁻⁷⁰. In sarcoidosis, SAA appeared to be increased in patients with active disease⁶³. To date, one of the strongest advantages of SAA above CRP has been demonstrated in kidney transplantation patients on immunosuppressive steroid therapy. In this later population only SAA appeared to be useful in monitoring acute rejection reactions, because in contrast to CRP, SAA expression is not influenced by corticosteroids^{59,71,72}.

For neither SAA or CRP, no diagnostic accuracy studies or studies aimed to predict fatigue in sarcoidosis have been reported by now.

High intensity alveolitis

Activation of T cells leads to the expression of IL-2 receptors on the cell surface and induces release of soluble IL-2 receptor (sIL-2R) molecules into the circulation. Besides, locally in the lung the redistribution of the cell-types occurs with increase in CD4-cells in the BAL.

Soluble IL-2R

Proliferation of T cells is controlled mainly by IL-2 and the recently discovered IL-15⁷³. For messages to be transmitted from cell to cell, IL-2 must bind to the IL-2R on the receiving cell. The receptor is expressed only on activated T cells and consists of three transmembrane protein chains: α (CD25), β (CD122), and γ (CD132). The α -subunit must associate with the β -subunit to form the IL-2 binding site. Binding of IL-2 with the receptor transduces a signal across the cell membrane and triggers T-lymphocytes to undergo clonal expansion⁷³⁻⁷⁷.

The α -subunit of the IL-2R is expressed on activated T cells and at lower levels on activated B cells, natural killer cells, and tumor cells^{76,77}. The IL-2R immunoassay is designed as an assay to measure this α (CD25) subunit (sometimes also called anti-Tac assay), as a soluble part of the IL-2R complex receptor (sIL-2R).

Increased levels of sIL-2R have been observed in lymphoproliferative disorders, neoplastic diseases and a variety of inflammatory and immunological disorders such as idiopathic pulmonary fibrosis, tuberculosis, leprosy etc.⁵⁰

It has been suggested that sIL-2R might be useful in monitoring pulmonary as well as extrapulmonary involvement in sarcoidosis^{34,35,50,77}. However, no appropriate diagnostic accuracy studies have been performed yet.

Granuloma formation

Angiotensin converting enzyme

Angiotensin I-converting enzyme (ACE), is a dipeptidyl-carboxypeptidase, mostly present on the luminal surface of the vascular endothelium.

The product of the enzymatic action of ACE is angiotension II, which is chemotactic for macrophages and may enhance the phagocytosis and accessory function of alveolar macrophages in sarcoidosis⁵⁰.

Physiologically, ACE is the key-enzyme of the renin-angiotensin system. Angiotensin II is a potent vasoconstrictor, which also stimulates adrenal production of aldosterone (sodium ion retention). Furthermore, ACE is able to cleave vasodilator bradykinin and some other neuronal peptides, inactivating them thereby^{50,78}.

Angiotensin II regulates cellular proliferation, inflammation and endothelial function through the activation of reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases⁷⁹. Namely, angiotensin II activates NAD(P)H-dependent oxidases and these form the most important vascular source of superoxide (O_2^-)^{80,81}, which stimulates nuclear factor- κ B (NF- κ B) to induce proinflammatory cytokines^{79,80}. The NF- κ B appears also to play a role in sarcoidosis⁸²⁻⁸⁴.

In sarcoidosis, ACE is mainly produced by activated granuloma cells (epithelioid cells). Therefore, elevated serum ACE concentrations reflect the total body granuloma burden. However, serum ACE correlates only roughly with the number of organs involved and the number of extrapulmonary sites as assessed by gallium scans⁵⁰. Besides ACE concentrations appeared to have poor predictive value in sarcoidosis^{34,85-87}.

There might be several reasons for that. Firstly, its less good performance might, at least for a part, be explained by the fact that ACE concentrations can be influenced by ACE polymorphism (I/D polymorphism in intron 16 of the ACE gene)^{88,89}. Therefore, adjustment of the reference values for the ACE polymorphism has been suggested^{89,90}. Nevertheless, so far, with respect to ACE polymorphism and susceptibility to disease progression, inconclusive data have been reported^{91,92}.

Secondly, ACE increase can be due to familiar ACE increase⁹³⁻⁹⁵. Thirdly, ACE increase is common in many other diseases, which explains its low specificity for sarcoidosis⁷⁸.

In spite of all of these limitations, ACE is still the only serologic parameter recommended by WASOG in sarcoidosis⁴. ACE is stated to be valuable in several

aspects: confirmation of suspected diagnosis, estimation of granuloma burden of the organism and follow-up of the disease during treatment⁴.

Oxidative stress and sarcoidosis

General facts about oxidative stress

Reactive oxygen and nitrogen species (ROS and RNS) are ubiquitous compounds produced by several types of reactions that can cause extensive damage to lung tissue. Enhanced ROS production was also demonstrated in sarcoidosis^{96-101,102}. The ROS include superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^{\cdot}). Among various RNS, one of the most toxic species is peroxynitrite ($ONOO^-$), derived from the reaction of $O_2^{\cdot-}$ and the nitric oxide radical (NO^{\cdot})¹⁰³⁻¹⁰⁵. The NO^{\cdot} is produced in high concentrations by the inducible nitric oxide synthase (iNOS), at sites of chronic inflammation.

During inflammation, ROS are produced through the process of so-called respiratory burst. The respiratory burst is an accelerated and increased O_2 uptake by neutrophils and macrophages, which can be ten to twenty times the “resting” O_2 consumption of neutrophils¹⁰⁴. The O_2 uptake is due to the activation of the NADPH oxidase enzyme complex. The activated complex oxidizes NADPH into $NADP^+$ in favor of the reduction of O_2 into $O_2^{\cdot-}$.

In the respiratory burst myeloperoxidase (MPO) and eosinophilic peroxidase (EPO) are important enzymes involved, which produce very potent oxidant hypochlorous acid (HOCl), used for microbial killing.

MPO is an abundant protein of neutrophils, stored in the azurophilic granules of neutrophils and released during phagocytosis^{106,107}. Important lung enzymes like acetylcholinesterase, glutathione-S-transferase and alpha-1-antitrypsinase can be modified by MPO in a way that they lose their enzymatic properties¹⁰⁸. An overview of the relationship between the most prominent reactive oxygen or nitrogen species is given in Figure 1.2A.

Under normal conditions, toxic oxygen metabolites are generated at a low level in lung cells by the transfer of a single electron during aerobic metabolism (respiratory chain for ATP production) in mitochondria.

Enzymatic systems such as NADPH oxidase, cytochrome P-450, monoamine oxidase, lipoxygenase and arachidonic acid metabolism represent another type of source of toxic oxygen species.

Lung antioxidants

The lung has to control the redox-balance in order to maintain normal cellular function. To be able to do that, it possesses an integrated antioxidant system.

Superoxide-dismutase (SOD) is an ubiquitous enzyme which catalyses conversion of $O_2^{\cdot-}$ radicals to H_2O_2 . The toxic H_2O_2 can be converted into H_2O in an enzymatic reaction by catalase. However, a central mechanism for reduction of H_2O_2 is the glutathione system (GSH, reduced form; GSSG, oxidized form of glutathione) in a

reaction catalyzed by glutathione peroxidase (GPx). The reaction catalyzed by GPx complements catalase as a reducing system for H_2O_2 but exceeds catalase in its capacity to eliminate additional varieties of toxic peroxides. Therefore, the cells try to maintain a high intracellular GSH:GSSG ratio.

In principle, oxidative stress can result in adaptation or cell injury¹⁰⁴. The adaptation can result in up-regulation of the synthesis of antioxidant defense systems. Such an adaptation has been shown in sarcoidosis patients reflected through an increased expression of catalase and superoxide dismutase^{109,110}.

The enzyme glutathione reductase (GR) maintains high intracellular concentrations of GSH by reducing the oxidized form of glutathione, GSSG, back to the reduced form, GSH. For this reaction, the reducing equivalents are derived from NADPH¹¹¹⁻¹¹⁴. The major pathway to generate NADPH is the hexose monophosphate shunt (HMS). In this pathway, glucose-6-phosphate dehydrogenase (G6PD) is the first and rate-limiting enzyme^{115,116}. In erythrocytes this is the only pathway to generate the NADPH. An overview of the enzymatic antioxidants is given in Figure 1.2B.

Additional antioxidants present in the lungs include ceruloplasmin, transferrin, ascorbate (vitamin C), alpha-tocopherol (vitamin E), ferritin, other serum proteins, and small molecules such as bilirubin.

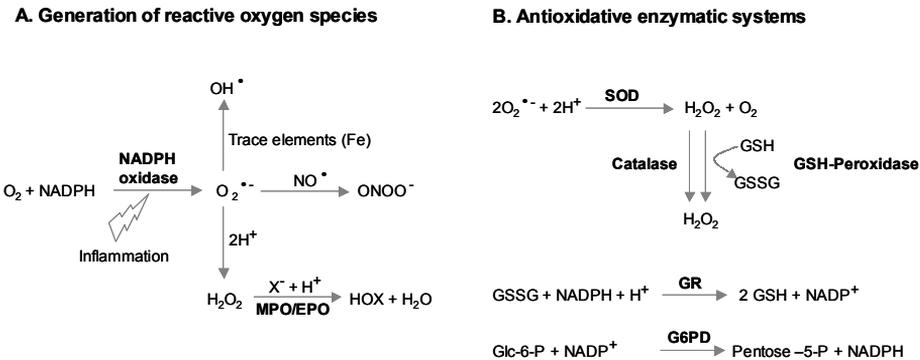


Figure 1.2. Generation of reactive oxidative species and the relation to antioxidative enzymatic systems.

$\text{O}_2^{\cdot-}$ = superoxide, H_2O_2 = hydrogen peroxide, OH^{\cdot} = hydroxyl radicals, ONOO^- = peroxynitrite, NADPH = nicotinamide adenine dinucleotide phosphate, MPO = myeloperoxidase, EPO = eosinophilic peroxidase, SOD = superoxide-dismutase, GSH/GSSG = glutathione system (GSH, reduced form; GSSG, oxidized form), GPx = glutathione peroxidase, GR = glutathione reductase, NO = nitric oxide radical, X^- = halide ion (Cl^- , Br^-), Glc-6-P = glucose-6-phosphate

Scope and aims of the study

The studies presented in this thesis were all performed in serum samples or in erythrocytes.

When features of sarcoidosis, for example radiological abnormalities and lung function impairment, resolve during treatment, disabling symptoms such as fatigue and pain may persist. Moreover, consensus about laboratory findings to depict disease activity or severity in sarcoidosis is still lacking. Overall this thesis concentrates on severity of sarcoidosis and the concept of activity has been left out from the evaluation.

The first aim of the studies presented in this thesis was to evaluate the clinical value of four different enzymatic markers, C-reactive protein (CRP), serum amyloid A (SAA), soluble interleukin-2-receptor (sIL-2R) and angiotensin converting enzyme (ACE). Firstly, the diagnostic accuracy of these markers to predict sarcoidosis severity was evaluated (CHAPTER 5). Secondly, the relationship of these markers and other clinical data with one of the major problems of sarcoidosis patients, *i.e.* fatigue, was studied (CHAPTER 6).

Prior to this, the methods used to measure these markers were evaluated and presented across CHAPTERS 2-4. In CHAPTER 2 and 3 the performance and agreement between the various CRP and hs-CRP methods were examined. In CHAPTER 4 the analytical performance and reference values for sIL-2R and SAA are presented.

The second aim of the studies presented in this thesis was to study several aspects of oxidative stress and its relationship with sarcoidosis.

Recently, it was hypothesized that oxidative stress might be involved in the pathogenesis of sarcoidosis. Among others, glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GR) are important antioxidant enzymes. These two enzymes are closely related. G6PD is the enzyme responsible for the regeneration of reduced nicotinamide adenine dinucleotide phosphate (NADPH). GR is involved in the regeneration of reduced glutathione (GSH), using the NADPH from the reaction catalyzed by G6PD. Indirectly, NADPH concentration can be measured by chromate-inhibition test. The state of these enzymes and indirectly the NADPH concentrations in the erythrocytes of the patients with sarcoidosis were analyzed in CHAPTER 7.

Previously, it was found that polymorphonuclear neutrophils might explain, at least in part, for severity in sarcoidosis. Myeloperoxidase (MPO) is considered as the most abundant enzyme of the neutrophils. Therefore, two functional polymorphisms, -463 G/A and -129 G/A, able to modify the expression of MPO were examined in sarcoidosis patients. Furthermore, their relationship with the severity of sarcoidosis was examined (CHAPTER 8).

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