Chapter 9

General discussion
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Sarcoidosis is characterized by an abundant immunological response, which in most cases spontaneously resolves. Nevertheless, in some cases the disease has a more or less severe course. Finally, it may lead to end-stage lung fibrosis with signs of respiratory functional impairment. One of the most important issues to deal with for clinicians is to identify those cases that will deteriorate and eventually develop fibrosis. Those cases should be monitored more closely and receive appropriate and timely treatment in order to prevent fibrosis development.

In general, the state of a disease can be classified either by activity or severity. Unlike other disorders, activity in sarcoidosis does not necessarily indicate a progressive course, a fatal prognosis, or the need for medical treatment. Many markers have been searched to reflect the disease activity. Unfortunately, the term “activity” has in the past been used unsystematically, resulting in the fact that no good marker of activity could be found. Previously, the term activity in pulmonary sarcoidosis has been related to the presence of T cell activation (alveolitis in case of pulmonary sarcoidosis), whereas until now no good consensus on activity exists (CHAPTER 1). Angiotensin converting enzyme (ACE) is still recommended by World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) as a marker of activity in sarcoidosis, despite of its shortcomings.

So far little attention has been paid to the appropriateness of markers to detect severity. From a clinical point of view, it is more important to depict whether the disease is severe, i.e. will have a more or less chronic course and will end-up with functional impairment rather than the initial activity. No proper marker of severity in sarcoidosis exists, which enables to predict deterioration. Moreover, this is complicated by the fact that an appropriate and sufficient definition of activity and severity is still lacking. There is a lot of discussion going on about the definitions of both terms, as it is difficult to make a clear distinction between these two entities. Besides, there is overlap between the two. Activity is a sign of inflammation. Severity, reflected through respiratory functional impairment, might be accompanied by inflammation (so activity may be present) as well. However, finally an end-stage situation without signs of an active inflammation can be found.

For each appropriate evaluation in the form of a diagnostic accuracy study for any marker a clear definition of the condition such as activity or severity is needed. In this thesis we introduce the definition of respiratory functional impairment to define pulmonary severity. We suggest the usage of this term, as it is as such of more interest to pulmonary physicians. Furthermore, we feel that severity, indicating functional impairment is of more clinical relevance.

Accordingly, one of the aims of this thesis was to search for new markers, which would enable to reflect disease severity in sarcoidosis. Respiratory functional impairment was used to depict the severity of the disease.

Four markers which have been claimed or suggested to be good markers of disease activity were evaluated for their ability to reflect disease severity: C-reactive protein
(CRP), serum amyloid A (SAA), soluble interleukin-2-receptor (sIL-2R) and angiotensin-converting enzyme (ACE). These markers were also related to one of the most common and bothering symptoms, \textit{i.e.} fatigue.

Prior to clinical evaluation of different biomarkers, the methods used to measure these markers were evaluated. A previous study showed that CRP was associated with metabolic derangement and fatigue in sarcoidosis, using our routine CRP method\textsuperscript{6}. CRP is also used as a marker of prediction of risk of future arteriosclerosis. From that aspect, several papers indicated that the routine CRP measurement as used among many laboratories could not be used in the low range because of the poor performance and too high detection limit (mostly between 2 and 5 mg/L). Moreover, in case of sarcoidosis, CRP changes were for a considerable part confined to the lower range\textsuperscript{6}.

Our routine CRP method (detection limit 5 mg/L) indeed appeared to suffer from serious shortcomings in the low range. Moreover, the method appeared to be extremely sensitive to sample turbidity, making the results obtained in such samples unreliable. This underlined further the need for a more appropriate method for the CRP measurement. The methods able to measure consistently very low CRP concentrations are called high-sensitivity CRP (hs-CRP) methods. Several hs-CRP methods were evaluated for their performance together with their sensitivity to turbidity of a sample. Based on the extensive evaluation of hs-CRP and CRP methods in \textit{CHAPTER 2} and \textit{3}, we decided to use BN ProSpec hs-CRP method for further evaluation of sarcoidosis patients.

Based on several previous studies it was assumed that SAA and sIL-2R might potentially be useful in sarcoidosis\textsuperscript{7-9}. We evaluated these two new assays for analytical performance and determined the reference values as shown in \textit{CHAPTER 4}. Evaluation of the sIL-2R method on the IMMULITE and the SAA method on the BN ProSpec yielded good imprecision results and satisfying linearity. The reference values for sIL-2R appeared to be sex and age independent. The reference values for SAA on BN ProSpec were sex dependent and showed a relationship with age.

The aim of the study presented in \textit{CHAPTER 5} was to evaluate the clinical usefulness of serological markers, of which the analytical performance was evaluated across the previous chapters. Thus, the diagnostic accuracies of hs-CRP, SAA and sIL-2R were established in sarcoidosis patients and they were compared to the traditionally used ACE in monitoring disease severity. Hundred forty-four non-smoking, 73 untreated (Group I) and 71 treated (Group II) patients were selected. The subgroups of the untreated patients, Group Ia (non-chronic group with time since diagnosis \(\leq 2\) years) and Group Ib (chronic group with time since diagnosis \(> 2\) years) were evaluated separately. ROC-curves and logistic regression analyses were used to compare the diagnostic accuracy of different markers to assess disease severity. Respiratory functional impairment \textit{i.e.} pulmonary disease severity was defined by means of lung function test results. The major finding of this chapter was that among the evaluated markers, in untreated patients, sIL-2R appeared to be the best marker to monitor
respiratory functional impairment and thus pulmonary severity. Furthermore, it appeared to be the most suitable marker for the follow-up. In line with others, sIL-2R was clearly shown to be superior to usually used ACE to monitor not only the activity but also pulmonary severity. However, the difference between the approach used in this chapter and other studies is that we performed an appropriate diagnostic accuracy study. For relationship between the markers, a fact that is often overlooked, was statistically corrected. Moreover, the value of the four markers was examined in an extensive way in non-treated as well as treated sarcoidosis patients. Finally, an attempt was made to give a reproducible and clear definition of criteria used to define pulmonary severity based on lung function test results as follows: DLCO < 80 or FVC < 80 or FEV1 < 80. We suggest that the criteria as defined in this chapter could be used more broadly; at least as long as there is a lack of official definition of severity criteria.

Patients with sarcoidosis most often suffer from symptoms related to the lungs, but may also suffer from a wide spectrum of other symptoms. One of the most often reported general symptoms is fatigue. As studies on the relationship between fatigue and laboratory and clinical parameters are sparse, in CHAPTER 6 this relationship was examined.

Previously it was shown that CRP appeared to be associated with metabolic derangement and fatigue in sarcoidosis, by means of less sensitive routine CRP measurement. Our evaluation in CHAPTERS 2 and 3 clearly showed that this routine method has serious shortcomings. Therefore, new hs-CRP method on the BN Prospec, the SAA and sIL-2R were measured among the serum markers in addition to traditionally used ACE. As factors that might predict fatigue also lung function test results reflecting respiratory functional impairment and the measures of metabolic derangement were included.

Patients with a time since diagnosis ≤ 2 years, (n = 60; 34 untreated, 26 treated) were clinically evaluated and completed the Fatigue Assessment Scale (FAS). It was confirmed that fatigue is a major problem in sarcoidosis. A representative sample of the Dutch control population (n = 1893) also completed the FAS. Only 27% of the sarcoidosis patients were classified as not being bothered by fatigue (FAS score < 22), compared to 80% in the control population. Moreover, in the sarcoidosis patients no sex differences and no differences in fatigue scores between the treated and the untreated groups were found.

The extent of fatigue was neither related to the new tested markers nor to ACE, metabolic derangement measures or lung function data. As long as there is a lack of ‘measurable’ clinical parameters to objectively assess fatigue, the FAS is useful and recommended as a supplementary tool to characterize the extent of fatigue in sarcoidosis.

The first part of this thesis was used to evaluate inflammatory markers and their ability to define respiratory functional impairment and fatigue in sarcoidosis. In the second part attention was paid to the systems, directly and/or indirectly involved in oxidative stress and microbial killing.
Prompted by earlier observations, it was hypothesized that oxidative stress probably plays an important role also in sarcoidosis and can eventually modify severity of the disease. Therefore, enzymatic systems, which take care of normal cellular redox-state glucose-6-phosphate dehydrogenase, G6PD and of continuous production of reduced glutathione (GSH) such as glutathione reductase (GR), were assessed in CHAPTER 7. The possibility was raised that some subclinical G6PD-deficiency might be present in patients with sarcoidosis that may modify disease severity. As the G6PD-gen lies on the X-chromosome, G6PD-deficiency will be clearly visible in males through the measurement of G6PD activity, but it will be masked in heterozygous females. Consequently, in females the degree of G6PD deficiency might vary enormously. Therefore, the chromate inhibition test (GR-Cr) as a more reliable test was used to assess the G6PD-deficiency. In the Netherlands, the GR-Cr test is the standard test used to establish the G6PD deficiency. This test is based on the inhibition of GR activity by chromate in erythrocytes with a normal level of reduced nicotinamide adenine dinucleotide phosphate (NADPH), but not in erythrocytes with decreased levels of NADPH, which might be a consequence of G6PD inactivity.

Decreased levels of NADPH, indicating G6PD deficiency, were found in 29% of the female sarcoidosis patients and in one male patient (2.5%). No explanation for this sex difference could be given. Six patients with abnormal chromate-inhibition test were chosen and their G6PD gene was completely sequenced, including non-coding and promotor region of the gene. The selection of patients for sequencing was random, although representing as well extremely abnormal as nearly normal GR-Cr values. Only one female of Negroid origin appeared to have a mutation in the G6PD gene. This same patient also had enormously decreased G6PD activity, so that even based on solely G6PD activity the diagnosis of G6PD deficiency could be considered. In the remaining patients no mutation in the G6PD gene was found.

Taken together, the GR-Cr test appeared inappropriate to reflect G6PD-deficiency in patients with sarcoidosis, due to its high-degree of false-positivity. One explanation for these findings is that an increased consumption of reduced NADPH is involved in the inflammatory process triggered by oxidative stress in sarcoidosis. However, the false-positivity of the test might also be caused by the increased GR activity on its own, so that the inhibition is less efficient. The GR appears to be present in two forms, the active form, which is associated with flavine adenine dinucleotide (FAD) and the inactive form, not associated with FAD. GR is almost completely saturated with FAD in red cells in patients with severe metabolic disorders or G6PD deficiency. In line with that, increased GR activity with a high degree of saturation with FAD can be found in red cells of severe uremia, cirrhosis of liver and G6PD deficiency. In the young red cells (reticulocytes) GR activity can also be increased and is sometimes used to correct for the presence of young cells, which in general have the activities of the most enzymes increased.

The major problem associated with G6PD deficiency is hemolytic anemia. Hemolytic anemia is the decreased ability of red blood cells to transport oxygen throughout the body, which can have rather serious consequences. Many (oxidative) drugs, infections, or fava beans can cause this. When any one of these agents enters the red blood cell,
hemoglobin becomes denatured, thus destroying its function as the principle oxygen-carrying molecule.

Usually, G6PD deficiency is accompanied with rather large restrictions in drugs and nutrients, which may be taken to avoid hemolytic crisis due to G6PD deficiency. Therefore, it is very important to have a reliable test to establish the G6PD deficiency. As shown in CHAPTER 7, the GR-Cr test cannot be used to establish G6PD deficiency in sarcoidosis patients. The question, which arises, is whether the same test can be used in other “comparable” inflammatory conditions. Anyhow, a careful revision of the test is needed.

In CHAPTER 8, the role of another enzyme involved in non-specific immunity, related to oxidative stress, has been examined. It was previously demonstrated that the number of polymorphonuclear neutrophils (PMNs) in the bronchoalveolar lavage (BAL) fluid is useful to distinguish patients with a more favorable outcome from those having a more severe course of disease in sarcoidosis. The major content of neutrophils is the oxidant-generating enzyme, myeloperoxidase (MPO). Cellular levels of MPO can be influenced by functional promoter polymorphisms, -463 G/A and –129 G/A that might modulate disease severity. These two MPO promoter polymorphisms were investigated in 110 sarcoidosis patients and in 191 ethnically matched controls. For both polymorphisms no significant differences were found between sarcoidosis patients and healthy controls. Furthermore, no association was observed between both polymorphism, and the severity of sarcoidosis.
Directions for future research

The study presented in CHAPTER 8 showed that two functional polymorphisms, which were previously shown to be related to diseases with comparable clinical presentation, were not associated with sarcoidosis. Also other studies looked at many different polymorphisms. However, although some of them found an association\(^{15-19}\), many showed a lack of any association with sarcoidosis\(^{3,20}\). Therefore, the search for a combination of polymorphisms, which might predict the course and progression of the disease (severity) is warranted.

Recently, a new unrecognized neurological complication of sarcoidosis, small-fiber neuropathy has been demonstrated\(^{21}\). For some disorders the link between decreased redox-state and cellular changes has already been established, such as neurons or cardiomyocytes\(^{22-25}\). Sarcoidosis has been suggested to trigger an oxidative stress response as also indicated by an increased activation of nuclear regulatory factor-kappaB (NF-\(\kappa\)B)\(^{26,27}\).

The possibility exists that the decreased redox-state in patients with inflammatory disorders including sarcoidosis might contribute to nerve-cell changes, which in turn can lead to small-fiber neuropathy. This is an interesting area with great clinical implications, which should be further explored.

As shown in CHAPTER 6, although helpful, a questionnaire is always at least partly a subjective measure to evaluate fatigue. Therefore, other markers able to objectify fatigue should be explored. Sarcoidosis manifests mostly in active young adults of age 20 - 40. The possibility exists that a stressful life accompanied with prolonged cortisol production can somehow evoke the sarcoidosis appearance. Possibly some hormonal dysbalance can also be a potentially useful measure of fatigue in sarcoidosis and should further be explored.

In sarcoidosis, there appears to be extra-renal production (by granuloma) of active form of vitamin D. As the active form of vitamin D has influence on calcium metabolism, the calcium metabolism can largely be deregulated in patients with sarcoidosis\(^{28}\). The mechanisms behind this deregulation as well as the clinical impact are largely unknown and need further to be explored.

Finally, one of most interesting areas to be explored and of interest in sarcoidosis is proteomics, which offers the possibility to look at the whole spectrum of serum or BAL fluid proteins at the same time. The first step has been set by group of Grünewald \textit{et al.}\(^{29}\). The proteomics might offer the possibility to find markers that will be able to distinguish acute from chronic patients and possibly to predict patients which will deteriorate due to fibrotic changes from those who will not end up with fibrosis. This new approach might give answers to many questions, which the clinicians are facing for a very long time. This underlines once more the importance of cooperation between clinicians and laboratories to explore the insight in this whimsical disorder sarcoidosis.
References


