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Analysis of serum chitotriosidase as prognostic biomarker of Sarcoidosis

Doctoral Thesis

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Summary

Sarcoidosis is an etherogeneous disease with an highly variable clinical course, hence, the identification of novel biomarkers with prognostic value is a major topic of the ongoing research. In the last years our group focused on a chitinase, named chitotriosidase, produced by activated macrophages. This enzyme was proven to be overexpressed in patients with sarcoidosis than healthy controls as well as patients affected by tuberculosis and other interstitial lung diseases.

The aims of the present study were to investigate the sensitivity and specificity of chitotriosidase in a large population of sarcoidosis patients and to analyse enzyme concentrations in different disease phenotypes.

We enrolled 232 sarcoidosis patients of Caucasian race (144 female, mean age 61 ± 13.2 years; M±SD), 188 of them had previous follow-up adequate to be classified into subgroups according to Clinical Outcome Status (COS classification).

Mean chitotriosidase activity in serum of sarcoidosis patients was 156.15 ± 93.81 nmol/h/ml and 16.13 ± 13.27 nmol/h/ml in controls, showing a significant difference ($p<0.0001$).

A positive correlation was found between peripheral blood concentrations of chitotriosidase and serum levels of ACE.

Statistically significant difference in chitotriosidase activity was found among COS subgroups. The lowest concentrations of chitotriosidase were found in untreated patients in remission (COS-1), while the highest enzyme concentrations were found in symptomatic patients with persistent disease on steroids and nevertheless with functional deterioration in the last year (COS-9).

Serum chitotriosidase resulted a sensitive and reproducible biomarker of Sarcoidosis as well as a reliable bioindicator of disease severity.

Background

Definition of Sarcoidosis

Sarcoidosis is a still enigmatic inflammatory disease characterized by histological findings of noncaseating epitheloid cell granulomas.
The American Thoracic Society/ European Respiratory Society/ World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) statement issued in 1999 provided the following definition: “Sarcoidosis is a multisystem granulomatous disease of unknown aetiology. It commonly affects young and middle-aged adults and frequently presents with bilateral hilar lymphadenopathy, pulmonary infiltration, ocular and skin lesions. Other organs may also be involved. The diagnosis is established when clinicoradiological findings are supported by histological evidence of noncaseating epitheloid cell granulomas. Granulomas of known causes and local sarcoid reactions must be excluded.” (1)
The descriptive nature of this definition reveals the lack of knowledge on causative agent that leads to granuloma formation, as well as the preferential involvement of certain certain organ systems.

Pathology

Since the cause of sarcoidosis is unknown the histological appearance provided the basis for the first disease definitions. So according to Scadding and Mitchell “sarcoidosis is a disease characterised by the formation in all of several affected tissues of epithelioid-cell tubercles without caseation though fibrinoid necrosis may be present at the centre of a few, proceeding either to resolution or to conversion into hyaline fibrous tissue". (2)

This is the description of disease’s hallmark: a discrete, compact, noncaseating epithelioid cell granuloma, which is viewed as a battle fought on a genetically vulnerable terrain between an unrecognised antigen and a highly organised team of lymphocytes and macrophages. (3)

The central portion of the granuloma harbors predominantly CD4+ T lymphocytes, whereas CD8+ lymphocytes are mainly located in the peripheral zone. Sarcoid granulomas may develop fibrotic changes that usually begin at the periphery and advance centrally, leading to complete fibrosis and/or hyalinisation. (4)

The granulomas are distributed along the pulmonary lymphatics, in the pleura and septa and along pulmonary arteries, veins, and bronchi. This distribution along lymphatic routes is peculiar and helpful in distinguishing it from other forms of granulomatous pulmonary disease.

Epidemiology

Sarcoidosis occurs throughout the world, affecting both sexes, and all races and ages. The real burden of the disease is difficult to assess due to the variability of clinical presentation and as a large percentage of affected individuals may never develop symptoms for a lifetime.

Therefore reported prevalence varies depending on which epidemiological method of selection is used with higher rates found in autopsy studies followed by mass chest radiography screening where prevalence varied from 0.2 per 100,000 individuals in Portugal to 64 per 100,000 screened individuals in Sweden (5).

Using a database from a Northwest USA population the incidence of clinically identified disease was estimated to be 4.8 per 100,000 (6).

With regard to sex and age, sarcoidosis has a preference for young adults with a peak incidence in males aged 30-34 years. Age distribution in females seems to be more complex as two peak of incidence were found at 25-29 years and at 65–69 years of age in a study based on Danish National Patient Registry data (7).

Northern European nations, notably Scandinavians, as well as Afro-Americans show the highest incidence rates in the world. In the latter group the disease also appears to be more severe.

Ethnicity is thought to exert influence on organ involvement as specific extrathoracic manifestations are common in certain populations, such as chronic uveitis in Afro-Americans, lupus pernio in Puerto Ricans and myocardial involvement in Japan. (8) (9) (10)
Overall mortality for sarcoidosis is up to 5% and it is commonly due to respiratory failure. (11)

Aetiology

Evidence from epidemiological and genetic studies as well as the immunological mechanisms behind the granuloma formation suggest that sarcoidosis could result from exposure of genetically susceptible hosts to specific environmental agents. (1)

Clustering of disease has been described and led to conjectures in the search for causative antigen(s). For example geographical clustering revealing that sarcoidosis occurs more often where lumbering and wood milling is a principal industry suggesteding a role of pinewood dust exposure. (12) In a case-control study conducted in the Isle of Man a huge proportion of sarcoidosis cases were reported prior contact with a person known to be affected by the same disease, raising the hypothesis that sarcoidosis is a communicable disorder or alternatively that patients might have shared a common occupational/environmental exposure (13) (14). A number of observational studies including the ACCESS study in USA found positive association between sarcoidosis and specific occupations but frequently selection biases are difficult to be ruled out and not so rarely sarcoidosis can represent a misdiagnosis of another antigen-induced environmental disease (15) (16).

Epidemiology also provided initial evidence of genetic predisposition to develop sarcoidosis as race is a well established risk factor and familial clustering has been observed (17). A growing number of studies were conducted to identify areas of genome linked to the disease. Many authors report linkage to a section within major histocompatibility complex (MHC) on the short arm of chromosome 6 and associations between class II MHC alleles and disease susceptibility or phenotypes (18) (19).

Also MHC based non-HLA genes could have a role as TNF-308A allele has been shown to be consistently associated with Löfgren's syndrome across different populations in northern Europe (20) and more recently butyrophilin-like 2 gene (BTNL2) was found to be associated with sarcoidosis independently from HLA-DRB1 (21).

Although no microbiological agent has been consistently cultured from sarcoidosis specimens, an infectious cause has been suspected for a long time and supported by some data on recipients of transplants from affected donors (22). This hypothesis seems quite logical considering that other granulomatous diseases such as Whipple's disease and tuberculosis were ultimately recognized as infectious diseases. In the past several microorganisms have been implicated implicated in the disease pathogenesis including viruses, *Propionibacterium acnes* (23), *Borrelia burgdorferi* and still today the search for a transmissible agent has been renewed thanks to modern molecular biology techniques, as well as immunohistochemical and electron microscopic examinations. Propionibacterial and mycobacterial rRNA were detected on sarcoidosis tissue samples (23) (24) but results were not yet reproducible, moreover ubiquitous nature of suspected bacteria makes difficult to prove causation.

Immunopathogenesis

In the last decades, research based on modern biotechnologies led to significant progress in our understanding on cell-mediated immune responses involved in the immunopathogenesis of sarcoidosis (25). The granulomatous response is thought to be initiated by the presentation of still unknown antigenic peptides in the context of MHC to the T-cell receptor (TCR). Activated CD4+ T lymphocytes are subsequently polarised to T-helper type 1 (Th1) cells under the influence of interleukin (IL)-12 and -18. Dendritic cells may also play a role in this polarisation process from Th0 into Th1 effector cells. Th1 lymphocytes play the crucial role in the second phase once they recognize the antigen presented by lung macrophages and start production host chemokines and cytokines to coordinate recruitment, migration, retention and local proliferation of cells,

especially T-lymphocytes and monocytes/macrophages involved in granuloma formation.

The third phase involves the evolution of the granulomatous inflammation, which can be classified as either spontaneous resolution or persistence of disease. Possibly the former depends on clearance of the causative antigen, as it happens in infectious granulomas, and requires a general downregulation of the immune response. Alternatively fibrotic changes occur when granulomatous inflammation persists. In a recent model proposed by Moller and Chen a shift in Th1/Th2 balance was found to be critical to enact an effective humoral response, which could help the clearance of pathogenic antigens (26).

Natural history and phenotyping

The clinical presentation and natural history of Sarcoidosis are highly variable. Incidental diagnosis in asymptomatic individuals is quite common, alternatively patients refers nonspecific constitutional symptoms or manifestations related to specific organ involvement. Spontaneous remission occurs in a large proportion of cases, and is particularly expected after acute onset of Löfgren Syndrome (fever, bilateral hilar adenopathy, erythema nodosum, and artralgia). Chronic or progressive course is seen in 10-30% (1). When significant or progressive pulmonary symptoms or extrapulmonary involvement is present corticosteroid treatment is usually started leading to stabilization or improvement in most cases, although relapses may occur in 16 to 74% of patients as the dose is tapered or discontinued (1).

Given the heterogeneity of clinical expression and prognosis it would be notably useful to find reliable indicators of clinical outcome.

Modern techniques in human genetics and molecular biology improved our ability identify genetic risk profiles for specific phenotypes. To this end, we need to define clinical phenotypes that predict clinical course and consequently provide a guide for management.

Historically a prognostic value was attributed to Scadding's roentgenographic staging (tab. 1): In Stage I disease, chest radiographs usually improve

spontaneously or stabilize while morbidity and mortality are most common among patients with chronic parenchymal infiltrates (Stages III and IV) (27) (28). This classification does not consider all the extrathoracic localizations.

In 2006, Wasfi et al. proposed a phenotype classification that included demographic data, lung function test results and organ involvement. This classifcation did not consider the disease evolution (29). In 2007 Prasse et al. (30) proposed a more dynamic classification (sarcoid sarcoid clinical activity classification, SCAC) based on three main clinical criteria: acute versus non-acute onset, need for treatment, request of long-term treatment (tab. 2).

More recently Baughman and the WASOG task force formulated a new phenotype classification based on clinical data and need of therapy during an observation period of almost 5 years, denominated “Clinical Outcome Score” (COS) (31). Patients are segregated into nine groups. At the beginning they are subdivided into the general categories of resolved, minimal disease arbitrarily defined as less than 25% of maximal disease, or persistent disease at five years. Further categorizing is then based on the use of systemic therapy: never treated, no therapy in the past year, and current therapy, which included patients treated in the past year. For those on current therapy, the patient is classified as being asymptomatic, symptomatic, or worsening over the past year. This led to a total of nine categories, as shown in Figure 1 (31).

Biomarkers

The unpredictable clinical course of sarcoidosis has prompted research into potential biomarkers that could help to predict disease outcome and support diagnosis. Among the most studied there are serum angiotensin-converting enzyme (ACE), Lysozime, cytokines and chemokines including soluble Interleukin-2 receptor (sIL-2R), markers of oxidative stress, bronchoalveolar lavage cell profile, CD4/CD8 ratio and chitotriosidase (32).

After many years of research, no single marker sufficiently sensitive and specific for diagnosis of sarcoidosis has yet been found. Serum angiotensin-converting enzyme (ACE) is the first widely used marker of sarcoidosis, but its

concentrations in serum and bronchoalveolar lavage (BAL) seem to have poor predictive value. T lymphocyte CD4/CD8 ratio in BAL has been recognized as an aid for diagnosis of sarcoidosis (33), although it has some limits. With regard to prognosis, the best evidence of clinical correlations is for sIL-2R and chitotriosidase, which seem more promising than ACE (34) (35). Other mediators, such as TNF-a and CCL18, could help to identify patients at risk for fibrosis or progressive disease (36) (37).

Chitotriosidase, also known as chitinase 1, is a member of family 18 of glycosylhydrolases, enzymes involved in the degradation of chitin and chitin-like substrate (38). The physiological functions of this enzyme are still unclear but there is some evidence that chitotriosidase is a component of innate immunity and takes part in defense against chitin-expressing pathogens, such as fungi, nematodes and insects (39). The enzyme is expressed by activated macrophages and elevated activity has been detected in serum of patients with atherosclerosis, b-thalassemia, acute Plasmodium falciparum malaria, and visceral leishmaniasis, as well as in cerebrospinal fluid of patients with multiple sclerosis (40). Chitotriosidase is considered to be a selective marker of macrophage activation (41) and the a biochemical marker of Gaucher's disease (42). In 2004 our group found significantly higher levels of chitotriosidase in serum of patients with sarcoidosis than in controls (43), subsequent studies suggested a possible role as a biomarker of disease severity as positive correlation was found between chitotriosidase activity in BAL and radiographic stages, serum levels of ACE, soluble interleukin-2 receptor (sIL-2R) and quantitative HRCT score (34) (44).

The reliability of chitotriosidase as a marker of sarcoidosis has been evaluated by detecting it in serum of patients with other granulomatous diseases, such as pulmonary tuberculosis, and different interstitial lung diseases, including pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis (45) (46). Interestingly, chitotriosidase was significantly higher in serum of sarcoidosis patients but not in other interstitial or granulomatous lung diseases with respect to controls (46).

Although the role of chitotriosidase in the pathogenesis of sarcoidosis is not yet clearly understood, it is reasonably that the enzyme secreted by activated macrophages may induce overexpression of profibrotic Th-2 cytokines (46).

Study presentation

Aim of the study

Sarcoidosis is an etherogeneous disease with an highly variable clinical course, hence, the identification of novel biomarkers with prognostic value is a major topic of the ongoing research. In particular in the last years our group of research focused on a chitinase, named chitotriosidase, produced by activated macrophages. This enzyme was proven to be overexpressed in patients with sarcoidosis than healthy controls as well as patients affected by tuberculosis and other interstitial lung diseases.

The aims of the present study were to investigate the sensitivity and specificity of chitotriosidase in a large population of sarcoidosis patients and to analyse enzyme concentrations in different disease phenotypes.

Patients and methods

Subjects

232 sarcoidosis patients of Caucasian race (144 female, mean age 61 ± 13.2 years; M±SD) were enrolled in the study; 195 were non-smokers, 20 current smokers and 17 ex-smokers. Controls were 70 Caucasians (43 female, mean age 52 ± 11.3 years); 46 were non-smokers, 14 current smokers and 10 ex-smokers.

All sarcoidosis patients were monitored regularly at the Sarcoidosis Regional Centre in Siena. Sarcoidosis was diagnosed according to international ATS/ERS/WASOG criteria (1). All subjects gave their written informed consent to the study, which was approved by the Local Ethics Committee. The patients underwent clinical, radiological and respiratory function tests including single-breath diffusing capacity for carbon monoxide. They performed chest x-ray in

posterior-anterior and lateral projections, classified by a single experienced radiologist. Radiological staging was performed in a standard manner according to widely accepted criteria (47): stage 0 - normal; stage 1 - bilateral hilar adenopathy; stage 2 - bilateral adenopathy and parenchymal infiltration; stage 3 - parenchymal infiltration; stage 4 - pulmonary fibrosis. Patients with hereditary chitotriosidase deficit (genetic polymorphism) were excluded, as well as patients with co-morbidities (such as lysosomal storage diseases, systemic sclerosis, beta-thalassemia, acute *Plasmodium falciparum* malaria and visceral *Leishmaniasis*) associated with elevated chitotriosidase levels.

The number of patients with an adequate follow-up (at least 5 years) was 188/232. They were classified in subgroups according to clinical outcome status (COS classification) (31): remission, minor involvement (defined as less than 25% of maximal disease) or persistent. These patients were further classified into nine categories on the basis of their need for systemic therapy: never treated, no therapy in the last year and need of systemic therapy in the last year. Patients on systemic therapy were divided into asymptomatic, symptomatic and deterioration in the last year. Patients in complete remission, not requiring therapy in the last year and never treated were classified as COS-1, and those not requiring therapy in the last year but previously treated as COS-2. Those with minor involvement (defined as less than 25% of maximal disease considering chest x-ray, respiratory function and involvement of other organs), not needing therapy in the last year were also divided into COS-3, never treated, and COS-4, not treated in the last year. Patients with persistent disease not on systemic therapy were divided into COS-5, never treated, and COS-6, not treated in the last year. Patients with persistent disease treated systemically were divided into asymptomatic patients without clinical functional deterioration in the last year (COS-7), symptomatic patients without clinical functional deterioration in the last year (COS-8) patients with clinical functional deterioration in the last year (COS-9) (31).

Bronchoalveolar lavage

Bronchoalveolar lavage was performed for diagnostic and clinical purposes with the informed consent of patients, by the standard procedure (48). Patients were not on pharmacological therapies at the time of bronchoscopy and BAL. BAL was obtained by instillation of four 60-ml aliquots of saline solution by

fibrobronchoscope (Olympus IT-10). The first sample was kept separate from the others and was not used for immunological tests. Cells were separated by centrifuge and the fluid fraction was frozen for enzyme assays. Cell differential counts were performed. Lymphocyte phenotype was analysed by flow cytometry (Facs-Calibur Becton & Dickinson) using anti-CD3, CD4 and CD8 monoclonal antibodies (Becton & Dickinson). BAL samples were cultured for microbes, fungi and viruses to exclude infections.

Chitotriosidase assay

Blood samples were collected in the morning after 8-hour fasting and were stored at -80°C until chitotriosidase assay. Chitotriosidase activity was determined by a fluorimetric method using 22 μM 4-methylumbelliferryl β D-NNN-triacetylchitotriosidase (Sigma Chemical Co.) in citrate-phosphate buffer, pH 5.2; 100 μl substrate was incubated for 1 h at 37°C and the reaction was stopped with 1.4 ml 0.1 M glycine-NaOH buffer, pH 10.8, as previously described (30,31). Fluorescence was read at 450 nm with a Perkin Elmer LS40 fluorimeter (excitation wavelength 365 nm). Serum levels of chitotriosidase were expressed in nmol/h/ml.

ACE assay

Serum concentrations of ACE were determined by a commercial assay (Buhlmann ACE colorimetric, Buhlmann Laboratories AG, Schonenbuch, Switzerland) generally used to determine ACE activity in serum, urine and tissues, as previously described (43).

Statistical analysis

Data was expressed as mean ± standard deviation (M ± SD). Comparisons between groups were performed by T-test with significance set at $p<0.05$. The Pearson test was used to look for correlations between variables. Statistical analysis was performed using Statistica v7.0 software; ROC analysis and graphic representations of data were conducted using GraphPad Prism Version 4.0 software for Windows.

Results

Age and gender of sarcoidosis patients and controls are indicated in Table 3 together with serum concentrations of ACE, pulmonary function test values (FEV1, FVC and TLCO), Scadding radiological stages and BAL features. Same data segregated into subgroup according to COS classification are shown in Table 4. Comparison of patients and controls revealed statistically significant differences in BAL lymphocyte percentages ($p<0.01$) and CD4/CD8 ratio ($p<0.05$).

Mean chitotriosidase activity in serum of sarcoidosis patients was 156.15 $\pm$ 93.81 nmol/h/ml and 16.13 $\pm$ 13.27 nmol/h/ml in controls, showing a significant difference ($p<0.0001$) (figure 2). ROC curve analysis revealed 0.98 area under the curve, 88.79% sensitivity (CI 84.01%-92.55%) and 92.86% specificity (CI 84.11%-97.64%) with a cut-off of 48.8 nmol/h/ml (figure 3).

A positive correlation was found between peripheral blood concentrations of chitotriosidase and serum levels of ACE (Pearson $r=0.25$, $p<0.001$) (figure 4). This correlation was especially evident in patients with chest x-ray stage 3 sarcoidosis ($r=0.45$, $p<0.001$). Chitotriosidase concentrations were significantly higher in stage 3 (n=50) than in stage 0 sarcoidosis (n=92) ($p=0.02$).

The number of patients in remission (COS-1 or 2) was 39/186 (n=29 never treated and n=10 no treatment in the last year), whereas 15/186 patients had minor involvement (COS-3 or 4) (n=12 never treated and n=3 no treatment in the last year) and 132/186 patients had persistent sarcoidosis (COS-5 to 9) (n=17 never treated, n=11 no treatment in the last year, n=33 patients in treatment but asymptomatic with no functional deterioration in the last year, n=43 patients in treatment and symptomatic but with no functional deterioration in the last year, n=28 patients in treatment and symptomatic with functional deterioration in the last year). Chitotriosidase concentrations were significantly different in COS-1 and COS-5 ($p=0.001$), COS-6 ($p=0.02$), COS-8 ($p=0.002$) and COS-9 patients ($p=0.0001$). Statistical differences were also detected between COS-5 and COS-7 ($p=0.03$) and COS-9 patients ($p=0.006$) (figure 5). The lowest concentrations of chitotriosidase were found in untreated patients in remission (COS-1), while the highest enzyme concentrations were found in symptomatic patients with persistent disease on steroids and nevertheless with

functional deterioration in the last year (COS-9). When the COS-9 population and the time trend of chitotriosidase were examined in greater detail, these patients were found to have undergone an increase in steroid dose or introduction of new immunosuppressant therapy in conjunction with steroids, associated with a significant decrease in chitotriosidase concentrations. Figure 6 shows serum levels of the enzyme in COS-9 patients before (time-0) the change in therapy and six months (time 1) after this change ($p<0.001$).

A negative correlation between chitotriosidase levels and DLCO values ($r=-0.7$, $p=0.009$) was observed in COS-6 patients. In the COS-8 subgroup there was another negative correlation between age and chitotriosidase levels ($r=-0.3$, $p=0.04$).

Discussion

In this study chitotriosidase concentrations were evaluated in a group of sarcoidosis patients, classified according to clinical phenotype, and a group of healthy controls. Chitotriosidase activity proved significantly higher in sarcoidosis patients than controls with a very high statistical difference. ROC curve analysis indicated that this biomarker of sarcoidosis had higher sensitivity (88.7%) and specificity (92.8%) that other bio-indicators of the disease: the sensitivity and specificity of ACE are about 68% and 75%, respectively, whereas those of soluble IL2 receptor are about 64% and 88%. Serum amyloid A sensitivity is very high 95% but it has a low specificity 35% (43).

The mechanisms leading to enhanced activity of chitotriosidase in serum of sarcoidosis patients is unclear but may be due to the abundant activated macrophages involved in the pathogenesis of sarcoidosis. Moreover, the enhanced activity of chitotriosidase in stage 3 patients and those with persistent progressive disease (in whom the development of chronic status could involve type 2 cytokines) suggest that it could be involved in T-helper 2 inflammation that also occurs in chronic sarcoidosis, as reported for other chitinases that take part in type2-inflammation through an IL-13-dependent mechanism (49).

The present study also aimed to evaluate chitotriosidase potential as a prognostic biomarker, demonstrating that chitotriosidase can be considered a bioindicator of sarcoidosis severity for the following reasons:

- its concentrations were significantly different in patients and controls;

- its serum concentrations showed a positive correlation with the concentrations of other sarcoidosis biomarkers (such as ACE) and an inverse correlation with functional parameters (such as DLCO) in COS-6 patients;
- a statistically significant difference was observed between chitotriosidase levels in patients with minimal disease compared with those with persistent progressive sarcoidosis;
- the highest concentrations of the enzyme were found in patients with persistent COS-9 sarcoidosis, while the lowest concentrations were observed in untreated patients with disease remission (COS-1);
- the introduction of new immunosuppressant therapies or the administration of steroid therapy in patients with severe persistent disease (COS-9) determined a significant decrease in serum levels of chitotriosidase.

In a previous paper, Tercelj et al. demonstrated that treatment with steroids caused a decrease in chitotriosidase activity in the majority of a population of 88 sarcoidosis patients (50). Brunner et al. analysed serum chitotriosidase levels in a small group of juvenile sarcoidosis patients before and after medical treatment, observing a reduction in chitotriosidase activity after introduction of oral steroid therapy (51). Also in our study, the effects of pharmacological treatment on chitotriosidase concentrations showed that patients with uncontrolled disease on steroids had very high enzyme levels that decreased drastically after steroid dose was increased or new immunosuppressant therapy was administered. These observations suggest that chitotriosidase can be a useful marker of the efficacy of therapy in sarcoidosis patients.

In conclusion, serum chitotriosidase resulted a simple, reliable and unexpensive biomarker of sarcoidosis patients. It was also much more sensitive and reproducible than other bioindicators of this disease. Its role in clinical practice (patient management and follow-up) could be suggested in order to differentiate stable from persistent progressive disease patients requiring closer monitoring. Chitotriosidase may be combined with other markers to determine severity, monitor patients in time and establish the efficacy of therapy.

Tables and Figures

TABLE 1. CHEST RADIOGRAPHIC STAGING OF SARCOIDOSIS (1)
0 Normal chest radiograph
I Bilateral hilar lymphadenopathy (BHL)
II BHL plus pulmonary infiltrations
III Pulmonary infiltrations (without BHL)
IV End-stage Pulmonary fibrosis

TABLE 2. SARCOID CLINICAL ACTIVITY CLASSIFICATION, SCAC (30)	
1	Acute onset, no need for immunosuppressive therapy
2	Acute onset, one period of treatment, not lasting longer than 1 yr
3	Acute onset, need for several periods of immunosuppressive therapy or long-lasting treatment (>12 mo)
4	Subacute onset, no need for immunosuppressive therapy
5	Subacute onset, one period of immunosuppressive treatment, not lasting longer than 1 yr
6	Subacute onset, need for several periods of immunosuppressive treatment or long-lasting treatment (>12 mo)

TABLE 3. Demographic characteristics, PFTs, BAL cell composition and chest x-ray in sarcoidosis patients and healthy controls. Significance is shown in the third column.

	Sarcoidosis	Controls	p
N° patients	232	70	
Age	61 ± 13.2	55 ± 11.3	ns
Sex	144 F	43 F	ns
Chitotriosidase	156. 15 ± 93.81	16.13 ± 13.27	<0.0001
ACE	34.65 ± 20.62 [1]	28.5 ± 20.77	ns
PFTs			
FEV1 %	99.3 ± 18.7	99.7 ± 10.3	ns
FVC %	105.6 ± 18.6	100.3 ± 8.2	ns
TLCO %	83.4 ± 16.4	89 ± 7.4	ns
TLCO/VA %	91.4 ± 15.9	97.1 ± 8.3	ns
BAL			
Macrophages	61 ± 16.4	85 ± 13.2	ns
Lymphocytes	36.6 ± 16.9	10.2 ± 8	<0.01
Neutrophils	2.7 ± 1.3	1.5 ± 1	ns
Eosinophils	2.8 ± 1.8	0.3 ± 3.1	ns
Mastocytes	0.75 ± 0.3	-	-
R CD4/CD8	4.4 ± 3.3	1.5 ± 2.2	<0.05
Chest XRay			
Stage 0	92 [2]	-	-
Stage 1	28	-	-
Stage 2	48	-	-
Stage 3	50	-	-
Stage 4	10	-	-

[1] positive correlation with serum chitotriosidase (r=0.25, p<0.001).
[2] positive correlation with serum chitotriosidase (r=0.45, p<0.001).

TABLE 4. Demographic characteristics, PFTs and BAL cell composition in sarcoidosis patients classified according to COS classification (n=186).

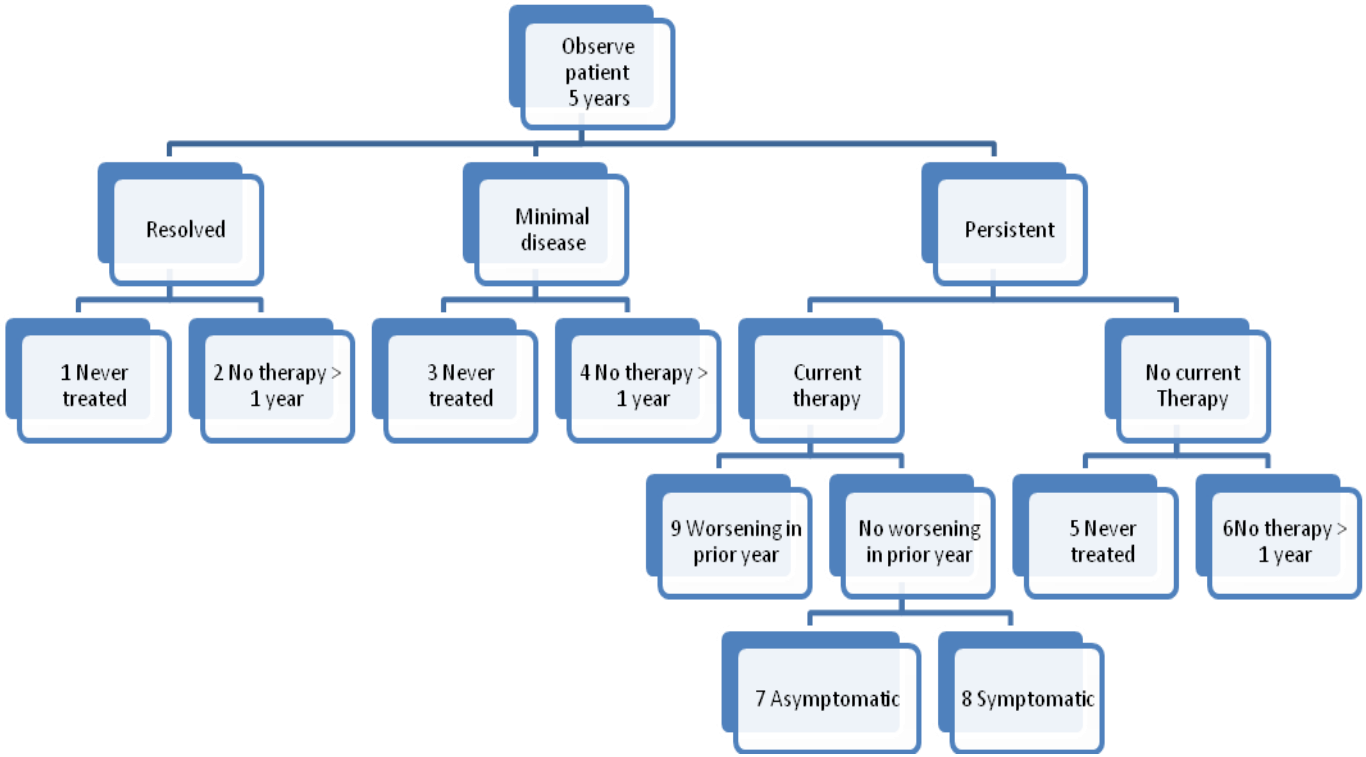
COS Classification	n (186)	Age	Sex	Chitotriosidase	ACE	FVC%	FEV1%	TLCO%	TLCO/VA%	Macr.	Lym.	Neu.	Eos.	Mast.	RCD4/CD8
COS 1	29	54 ± 17.4	18 F	91.43 ± 53.24 *	34.33 ± 19.59	109.6 ± 18.3	106.6 ± 16.4	89.3 ± 12.1	92.6 ± 14.6	-	-	-	-	-	-
COS 2	10	56 ± 12.6	9 F	97.67 ± 40.78	35.5 ± 12.8	117.8 ± 16	111.6 ± 21	90 ± 13.8	93.4 ± 8.2	60.5 ± 45.9	37.5 ± 45.9	2 ± 0	-	-	4.01 ± 3.9
COS 3	12	54.3 ± 10.4	6 F	154.5 ± 88.62	32.4 ± 12.9	97.6 ± 11.3	93.6 ± 15.4	93.1 ± 17.9	105.1 ± 13.3	-	-	-	-	-	-
COS 4	3	58 ± 14.7	3 F	120.2 ± 62.14	18 ± 19.7	106.6 ± 30.9	101 ± 28.5	78.6 ± 3.5	85 ± 1.4	73.5 ± 20.5	20.5 ± 12	2 ± 0	-	-	1.9 ± 1.8
COS 5	17	56.5 ± 15.7	10 F	197.6 ± 70.34*°	34.6 ± 17.8	106.9 ± 15.5	100.4 ± 15.6	83.4 ± 16.7	89.1 ± 15.1	65.6 ± 13.9	32.8 ± 12.6	2 ± 0	1.5 ± 0.7	-	5.29 ± 3.73
COS 6	11	54.1 ± 15.8	4 F	209 ± 117.14*	36 ± 23.1	91.3 ± 17.7	83 ± 20	72.6 ± 14.9 [1]	85.9 ± 18.2	-	-	-	-	-	-
COS 7	33	59.6 ± 13.6	21 F	116.98 ± 85.16°^	31.3 ± 12.4	113.4 ± 16.5	108 ± 16.7	85.5 ± 15.2	91.8 ± 13.2	63.6 ± 20	35.2 ± 20.8	2.2 ± 1.7	1.5 ± 0	-	6.19 ± 4.98
COS 8	43	59.3 ± 10.9 [2]	25 F	176.14 ± 95.25*	37 ± 22.9	99.1 ± 20.7	89.9 ± 18.9	78.7 ± 17	87.9 ± 18	53 ± 13.2	45.2 ± 15.79	3.8 ±1.4	4 ± 2.6	-	3.76 ± 3.31
COS 9	28	53.9 ± 15.1	17 F	196.93 ± 94.23*^	44.6 ± 31.4	100.1 ± 18.7	94.9 ± 17.1	77.2 ± 15.6	89.3 ± 16.7	56 ± 12.8	40.8 ± 12.5	4.2 ± 1.7	1 ± 0	-	5.39 ± 3.57

Significant differences were found between controls and all COS stages (p<0.001) (§). Chitotriosidase was significantly different in COS-1 than COS-5 (p=0.001), COS-6 (p=0.02), COS-8 (p=0.002) and COS-9 patients (p=0.0001) (*); in COS-5 than COS-7 patients (p=0.03) (°); and in COS-7 than COS-9 patients (p=0.006) (^).

[1] negative correlation with serum chitotriosidase (r=-0.7, p=0.009).

[2] negative correlation with serum chitotriosidase (r=-0.3, p=0.04).

Fig. 1



Phenotyping of Sarcoidosis patients according to “Clinical Outcome Score” (COS) (31)

Fig. 2

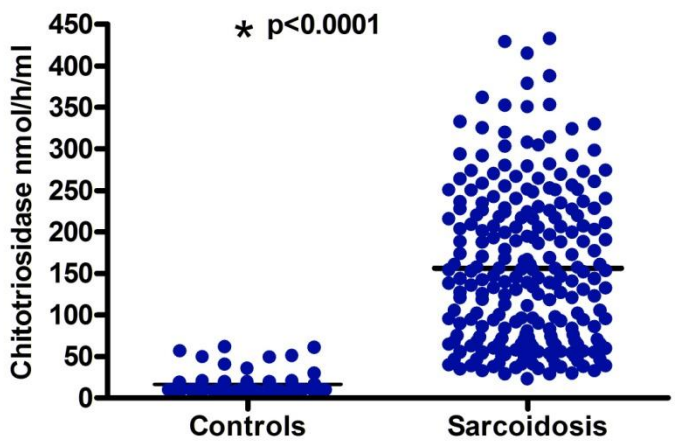
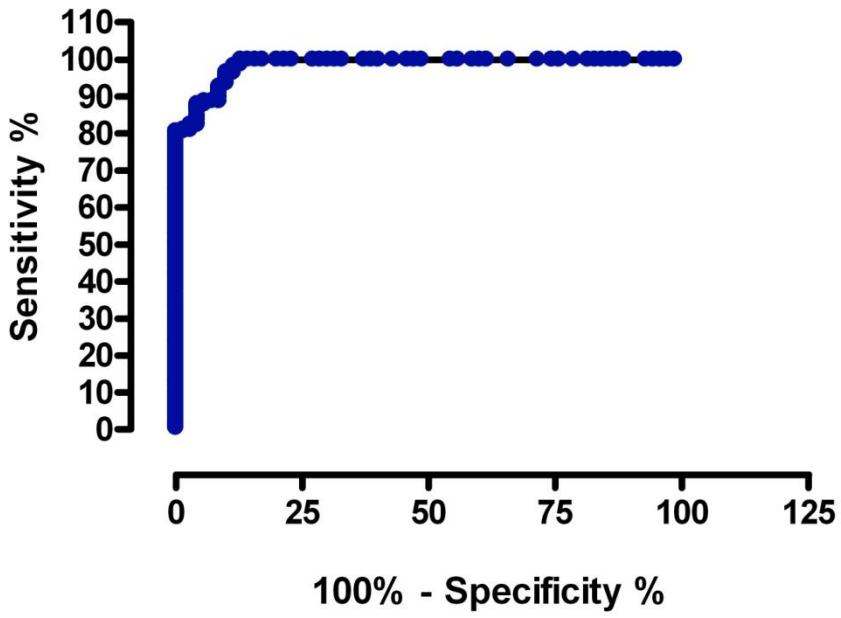


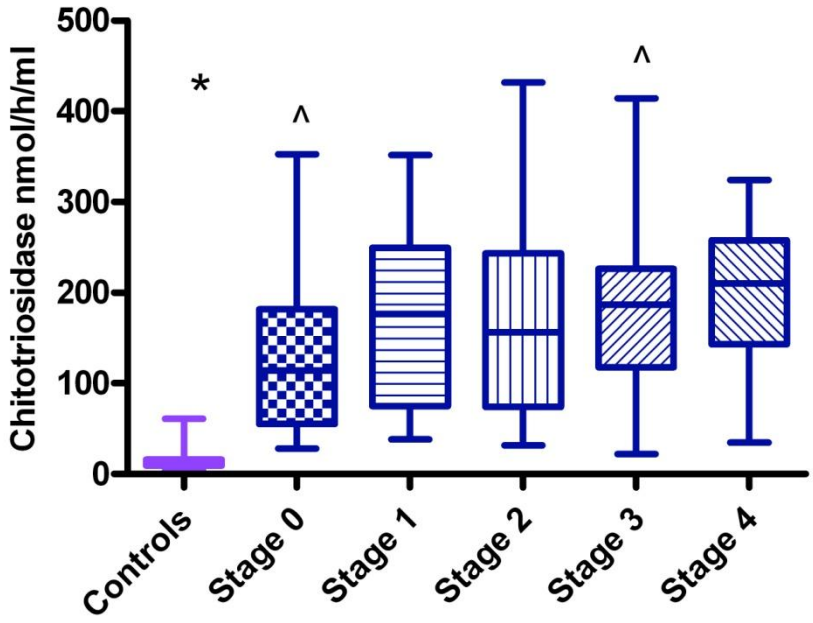
Figure shows chitotriosidase concentrations in controls (n=70) and in sarcoidosis patients (n=232) (p<0.0001).

Fig. 3



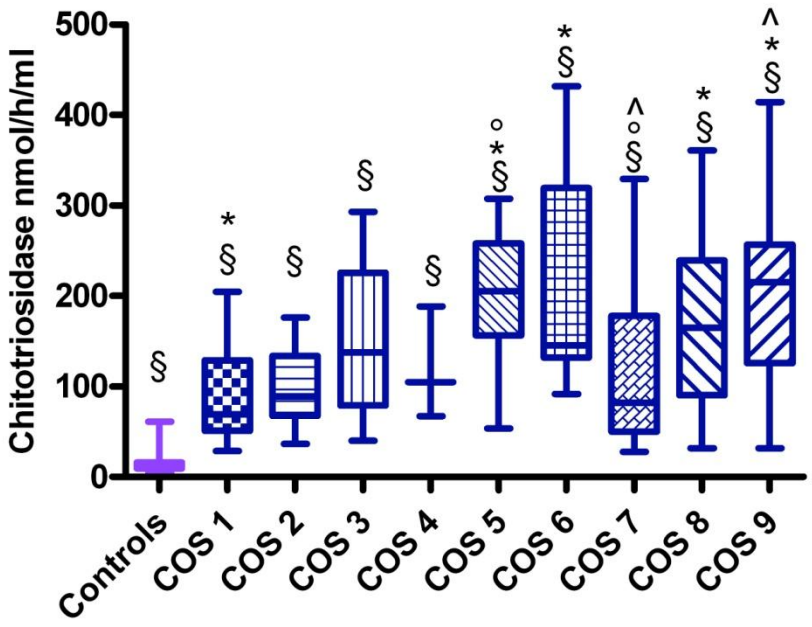
ROC (Receiver Operator Characteristic) curve showing specificity and sensitivity percentages of chitotriosidase in sarcoidosis patients (n= 232). Area under the curve 0.98, chitotriosidase cut-off value 40.20 nmol/h/ml, sensitivity 92.60%, specificity 91.40%, likelihood ratio 10.80.

Fig. 4



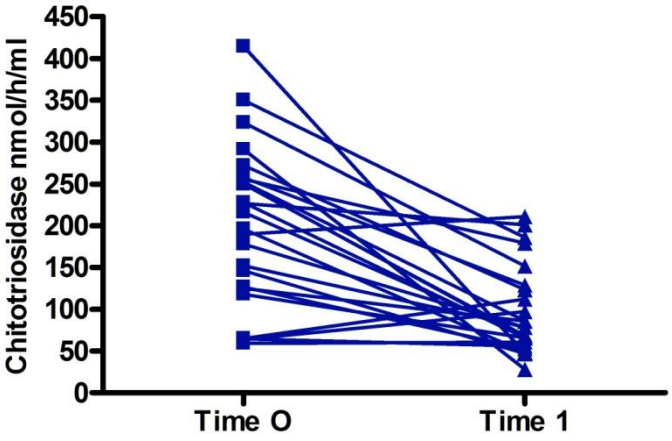
Chitotriosidase concentrations in controls (n=70) and sarcoidosis patients classified according to chest radiographic stages (n=232). Stage 0 n=92 chitotriosidase 128.7±83.2 nmol/h/ml, Stage 1 n=29 chitotriosidase 167.4±97.6 nmol/h/ml, Stage 2 n=49 chitotriosidase 172.8±106 nmol/h/ml, Stage 3 n=51 chitotriosidase 176.4±87.2 nmol/h/ml, Stage 4 n=11 chitotriosidase 188.3±94.1 nmol/h/ml. Chitotriosidase levels were significantly different in controls than in all Scadding stages ($p<0.0001$) (*).

Fig. 5



Chitotriosidase concentrations in COS subgroups (n=186). Significant differences were found between controls and all COS stages ($p<0.001$) (§). Chitotriosidase was significantly different in COS-1 than COS-5 ($p=0.001$), COS-6 ($p=0.02$), COS-8 ($p=0.002$) and COS-9 patients ($p=0.0001$) (*); in COS-5 than COS-7 patients ($p=0.03$) (°); and in COS-7 than COS-9 patients ($p=0.006$) (^).

Fig. 6



Chitotriosidase levels in COS-9 patients before changing therapy (time 0) and six months (time 1) after the change (n=25) ($p<0.001$).

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