Respiratory Medicine (2007) **101**, 2176–2181

ELSEVIER

respiratoryMEDICINE

# Chitotriosidase activity in patients with interstitial lung diseases

**Elena Bargagli[a,*], Maria Margollicci[b], Alice Luddi[b], Nicolaos Nikiforakis[a], Maria Grazia Perari[a], Salvatore Grosso[b], Anna Perrone[a], Paola Rottoli[a]**

[a]*Respiratory Diseases Section, Department of Clinical Medicine and Immunology, Siena University, Italy*
[b]*Department of Paediatrics, Siena University, Italy*

Received 27 October 2006; accepted 6 May 2007
Available online 13 July 2007

**KEYWORDS**
Chitotriosidase;
Serum;
Bronchoalveolar lavage;
Interstitial lung diseases;
Sarcoidosis;
Idiopathic pulmonary fibrosis

**Summary**
*Background:* In previous papers, we found significantly higher activity of chitotriosidase, a macrophage derived enzyme, in serum and BAL of patients with sarcoidosis, especially in those with progressing disease and lung involvement, than in controls. Locally and systemically produced chitotriosidase activity was correlated with radiological stage and also with degree of lung infiltration, suggesting that this enzyme may play a role in the pathogenesis of sarcoidosis and may be used as a marker of disease severity.
*Aim:* To analyse chitotriosidase activity in serum and bronchoalveolar lavage of patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with systemic sclerosis and to compare it with chitotriosidase activity in controls and sarcoidosis patients.
*Methods:* Chitotriosidase activity was determined by a fluorometric assay.
*Results:* The results showed that serum chitotriosidase activity was only elevated in sarcoidosis patients; in patients with idiopathic pulmonary fibrosis and pulmonary fibrosis associated with systemic sclerosis it was in the normal range. On the contrary, in BAL of sarcoidosis and idiopathic pulmonary fibrosis patients the activity was significantly higher than in controls.
*Conclusion:* Serum chitotriosidase is a potential marker of sarcoidosis severity; it increases in sarcoidosis in relation to radiological stage and degree of lung infiltration. The increase in chitotriosidase activity in BAL of sarcoidosis and idiopathic pulmonary fibrosis patients suggests that the enzyme could be involved in fibrogenesis in diffuse lung diseases. Further research is needed to understand the role of chitotriosidase in the pathogenesis of sarcoidosis and its involvement in fibrotic remodelling in certain diffuse lung diseases.


*Abbreviations:* BAL, bronchoalveolar lavage; ILD, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; SSc, pulmonary fibrosis associated with systemic sclerosis

*Corresponding author. Respiratory Diseases Section, Department of Clinical Medicine and Immunology, Policlinico Universitario, Le Scotte, Viale Bracci, 53100 Siena, Italy. Tel.: +39 0577 586710; fax: +39 0577 280744.
*E-mail address:* bargagli2@unisi.it (E. Bargagli).

0954-6111/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2007.05.008

## Introduction

Interstitial lung diseases (ILD) are a heterogeneous group of lung disorders with different clinical courses and pathogenetic mechanisms. Among these diseases, the most common are sarcoidosis (S), idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis associated with systemic sclerosis (SSc). The prognosis of these diseases depends on the different evolution towards lung fibrosis. IPF is rapidly progressive and unresponsive to therapy.[1,2] This chronic interstitial disorder is limited to the lungs and involves fibroblast proliferation, extracellular matrix deposition, tissue remodelling and fibrotic destruction of the lung. On the other hand, sarcoidosis is a multisystemic granulomatous disease generally associated with a good prognosis (although some patients develop chronic forms, such as pulmonary fibrosis). The etiopathogenesis of sarcoidosis and IPF are still unknown, although sarcoidosis is an inflammatory disease while IPF is secondary to epithelial lung injury.[2–5] The unpredictable clinical courses of these ILD have motivated research into potential prognostic biomarkers.[6–11]

Human chitotriosidase (also known as chitinase 1, a family 18 glycosylhydrolase) breaks down colloidal chitin and is inhibited by the family 18 chitinase inhibitor, allosamidin.[12] The newly synthesised 50 kDa enzyme consists of an N-terminal structure containing the catalytic groove and a C-terminal chitin-binding domain connected by a short hinge region.[13,14] Although the physiological functions of this enzyme are still unclear, there is evidence[15–17] that chitotriosidase is a component of innate immunity and may defend against pathogens containing chitin, such as fungi, nematodes and insects. Chitotriosidase is considered a marker of macrophage stimulation, being mainly produced by chronically activated tissue macrophages.[16,18–21] It was recently reported that leucocytes may also secrete plasma chitotriosidase under physiological conditions.[16,22] Increased activity of serum chitotriosidase is reported in patients with lysosomal storage disorders (Gaucher syndrome, fucosidosis, galactosialidosis), beta-thalassemia, acute *Plasmodium falciparum* malaria and visceral Leishmaniasis.[23–29] Increased chitotriosidase activity is also reported in cerebrospinal fluid of patients with multiple sclerosis.[25]

In the framework of our research into possible biomarkers of ILD, we reported a statistically significant increase in chitotriosidase activity in serum of sarcoidosis patients with respect to serum of controls for the first time in 2004.[29] The enzyme activity in serum was correlated with sarcoidosis radiographic stages, suggesting potential as a prognostic marker of sarcoidosis. No such correlation was found for serum levels of ACE. We then investigated alveolar expression of chitotriosidase in BAL and its reliability as a potential biomarker of sarcoidosis severity.[30] Chitotriosidase activity in BAL showed a positive correlation with radiographic stages, serum levels of ACE and quantitative HRCT score, especially in patients with progressing sarcoidosis. The hypothesis that serum chitotriosidase activity may be an indicator of sarcoidosis severity was further supported by recent research of ours into its activity in serum of patients with pulmonary tuberculosis and sarcoidosis. We only found increased activity of chitotriosidase in serum of sarcoidosis patients; those of TB patients and controls were similar.[31] Other studies are still necessary to determine whether chitotriosidase could be a useful marker of sarcoidosis severity.

In order to find out whether chitotriosidase is altered in other ILD, we assayed its activity in serum and BAL of IPF and SSc patients. The results were compared with those of serum and BAL of sarcoidosis patients and healthy subjects.

## Patients and methods

### Subjects and sampling

The study population consisted of 38 IPF patients and 20 SSc patients (Table 1): 7/38 IPF patients and 4/20 SSc patients were smokers. None of the patients had ever been treated with steroids or other immunosuppressants, they were regularly monitored at our Sarcoidosis and Interstitial Lung Disease Regional Referral Centre in Siena from onset for at least 12 months. They had no history of concomitant pathology. They gave their written informed consent to the study. IPF was diagnosed according to ATS/ERS international criteria.[1,2] Diagnosis of SSc was based on clinical–radiological and immunological findings and histopathological features. All patients underwent chest HRCT at onset and every 12 months, documenting fibrosis ranging from mild to moderate. The patients enrolled in the study had a documented history of lung fibrosis secondary to systemic sclerosis, of at least 18 months. Characteristics of sarcoidosis patients and criteria for differentiating disease severity are reported in previous papers.[29,30] Control subjects were matched for age and gender, had no history of asthma or allergy and were not under therapy of any kind. They had normal lung function parameters and chest X-ray. They were monitored for 12 months and did not develop any diseases. Only one control was a smoker. All subjects gave written informed consent to the study.

**Table 1** The anagraphic features of patients with idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis associated with systemic sclerosis (SSc) are reported together with the functional data, $PaO_2$ levels on blood gas analysis, desaturation on 6 min walking test and systolic pulmonary arterial pressure estimated by echocardiogram.

| | Age ($M \pm SD$) | Sex (M/F) | Smoke (n.pt./tot.) | CV (%) ($M \pm SD$) | FEV1 (%) ($M \pm SD$) | DLCO (%) ($M \pm SD$) | $PaO_2$ (mmHg) ($M \pm SD$) | Desaturation (n.pt./tot.) | PAPs (mmHg) ($M \pm SD$) |
|---|---|---|---|---|---|---|---|---|---|
| FPI (no. 38) | 63.35 ± 9.67 | 29/9 | 7/38 | 68.6 ± 24.75 | 74.13 ± 23.82 | 42.24 ± 12.36 | 64.20 ± 12.28 | 10/38 | 31.33 ± 14.93 |
| SSc (no. 20) | 55.05 ± 9.11 | 7/13 | 4/20 | 73.13 ± 30.89 | 73.7 ± 29.5 | 43.4 ± 38.18 | 70.01 ± 19.57 | 4/20 | 36.47 ± 22.88 |

Clinical, radiological and respiratory function tests including single-breath diffusing capacity for carbon monoxide were done in both group of patients (Table 1). Chest X-rays were performed in the posterior–anterior and lateral projections and were classified by a single experienced radiologist. High-resolution computed tomography of the chest was done and a single experienced reader described the scans. The following parameters were recorded: age, sex, BAL differential cell count, BAL lymphocyte phenotype, lung function test (FVC, FEV1, DLCO, TLC), oxygen desaturation during 6 min walking test, $PaO_2$ values by blood gas analysis, pulmonary arterial pressures estimated by echocardiogram (Table 1), radiological impairment by chest X-ray and chest HRCT. In the group of IPF patients the most common symptoms at onset were stress-related dyspnoea and dry cough; dyspnoea and asthenia were the most common symptoms in the SSc patient group.

Blood samples were always collected in the morning, after 8-h fasting and samples were stored at −80 °C until assay. Bronchoalveolar lavage was performed for diagnostic or clinical purposes with the informed consent of patients. Samples were obtained by instillation of four aliquots (60 ml each) of saline solution by a fibrobronchoscope (Olympus IT-10). The first BAL sample was kept separate from the others and was not used for immunological tests. Sub-samples were cultured for microbes, fungi and viruses to exclude infections. 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), as previously described.[32]

## Chitotriosidase assay

Chitotriosidase activity was determined by a fluorimetric method using as substrate 22 μM of 4-methylumbelliferyl-β-D-*NNN*-triacetylchitotrioside (Sigma Chemical Co.) in citrate–phosphate buffer, pH 5.2. 100 μl of substrate was incubated for 1 h at 37 °C and the reaction was stopped with 1.4 ml of 0.1 M glycine–NaOH buffer, pH 10.8, as previously described.[29–31] Fluorescence was read at 450 nm on a Perkin-Elmer LS40 fluorimeter (excitation wavelength 365 nm). BAL samples from patients and controls were not concentrated, each sample was assayed in three replicates. Serum levels of chitotriosidase were expressed in nmol/h/ml, while chitotriosidase activity in BAL was expressed as nmol/h/mg protein. Total protein concentrations in BAL were determined with the Bio-Rad Protein assay, based on Bradford's method.[33] Inter- and intra-assay coefficients of variability were, respectively, 3.7% and 1%.

## Statistical analysis

Data were expressed as mean ± standard deviation ($M \pm SD$). Since it was not normally distributed (Shapiro–Wilk normality test), the Kruskall–Wallis test was applied to compare significant differences between mean values. Comparisons between groups were performed by a non-parametric test, namely Dunn's multiple comparison test ($p < 0.05$). All variables were cross-correlated using the Spearman $R$ coefficient of correlation ($p < 0.05$). Statistical analysis was performed using GraphPad Prism Version 4.0 software for Windows (2005).

# Results

Anagraphic, clinical, functional features of the groups of IPF and SSc patients are reported in Table 1 and BAL cell profile and CD4/CD8 ratio in Table 2.

Figure 1 compares chitotriosidase activity in serum from IPF (19.9 ± 13.57 nmol/h/ml) and SSc (12.56 ± 3.93 nmol/h/ml) patients with previously published activities[31] in serum of 95 sarcoidosis patients (118.6 ± 114.4 nmol/h/ml) and controls (10.39 ± 2.83 nmol/h/ml). As previously described by us and according to the considerable amount of control data reported in literature (e.g. 15, 18, 26, 29), chitotriosidase activities < 18 nmol/h/ml are assumed to be normal values. Mean chitotriosidase activities in BAL samples from IPF and SSc patients (15.79 ± 17.25 and 8.92 ± 7.36 nmol/h/mg, respectively) were higher than previously reported values[30] in stable sarcoidosis patients (2.79 ± 1.49 nmol/h/mg) and control subjects (2.06 ± 1.36) and those of IPF patients were in the same range as those measured in patients with progressive sarcoidosis (17.52 ± 13.39 nmol/h/mg). SSc patients had values intermediate between those of progressive sarcoidosis and controls (Fig. 2).

Serum chitotriosidase activity was significantly lower ($p < 0.001$) in IPF and SSs patients than in sarcoidosis patients, whereas differences with respect the control group were not significant. The BAL samples from progressing sarcoidosis patients had chitotriosidase activities significantly higher than those from stable sarcoidosis patients and controls ($p < 0.001$). Values in IPF patients, but not SSc patients, were significantly higher than values measured in controls ($p < 0.05$) (Fig. 2).

No differences were found between chitotriosidase activity in serum and BAL of smokers and non-smokers, though the number of smokers was too small for statistical analysis.

Chitotriosidase activities in BAL of SSc patients were inversely correlated with the total number of BAL cells (Spearman coefficient of correlation $R = -0.806$, $p < 0.004$)

**Table 2** The total cell number on bronchoalveolar lavage, the bronchoalveolar lavage cell profile and CD4/CD8 ratio of BAL lymphocytes were reported in patients with idiopathic pulmonary fibrosis (IPF) and pulmonary fibrosis associated with systemic sclerosis (SSc).

| | n. tot. cell. ($M \pm SD$) | M (%) ($M \pm SD$) | L (%) ($M \pm SD$) | PMN (%) ($M \pm SD$) | EOS (%) ($M \pm SD$) | CD4/CD8 ($M \pm SD$) |
|---|---|---|---|---|---|---|
| FPI (no. 38) | 19.878.235.3 ± 19.444.346 | 50.52 ± 26.78 | 26.94 ± 23.58 | 15.65 ± 18.01 | 7.5 ± 8.6 | 1.7 ± 1.23 |
| SSc (no. 20) | 16.142.222 ± 9.632.389 | 69.7 ± 17.44 | 21.2 ± 15.03 | 8.38 ± 10.83 | 4 ± 2.44 | 1.33 ± 0.93 |

and chitotriosidase activities in IPF patient serum were inversely related to the percentage of neutrophils in BAL from IPF patients ($R = -0.84$, $p < 0.002$). A correlation ($R = 0.64$, $p > 0.04$) was also found between chitotriosidase activities and lymphocyte percentages in BAL samples from SSc patients.

## Discussion

Our results show serum chitotriosidase activities in the normal range (<18 nmol/h/ml) in patients with IPF and SSc. Measured activities were six to nine times lower than previously reported activities in serum of sarcoidosis patients. This result strongly supports our hypothesis that serum chitotriosidase activity could be a potential marker of sarcoidosis severity due to enhanced activation of macrophages during formation of sarcoid granulomas[34,35] or to the potential involvement of agents containing chitin in the development of the disease; while chitotriosidase levels in serum do not indicate any involvement of this chitinase in IPF or SSc.

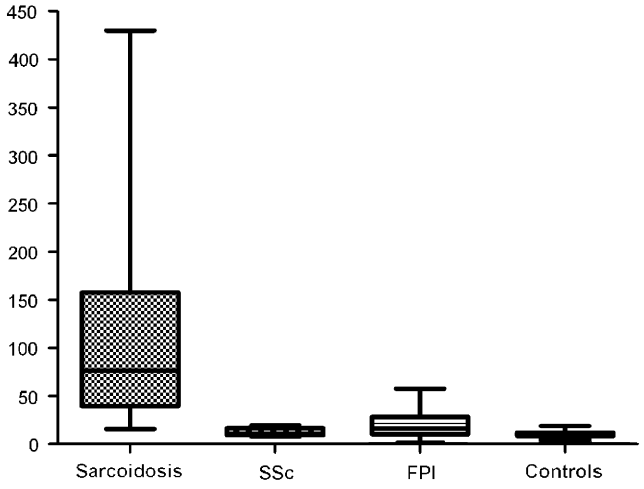


**Figure 1** Chitotriosidase activity (nmol/h/ml) in serum of patients with sarcoidosis, idiopathic pulmonary fibrosis, pulmonary fibrosis associated with systemic sclerosis and controls. The first line represents the minimum value, the second line is the 25° percentile, the third line is the mediane, the fourth line is the 75° percentile and the fifth line is the maximum value.

Interestingly, chitotriosidase activities in BAL showed a different pattern, being significantly higher in IPF and not only in sarcoidosis patients than controls (Fig. 2). This result suggests that the increase in chitotriosidase could be the expression of high activation of certain macrophage subsets responsible for remodelling and tissue damage both in S and IPF at alveolar level. A similar process has been described in non-alcoholic fatty liver disease.[36,37] Overexpression of chitotriosidase was recently reported to promote progression of uncomplicated steatosis to hepatic fibrosis.[36,37] Involvement of chitotriosidase in fibrogenic and remodelling processes also seems supported by the results of our previous studies. In fact, activity of chitotriosidase in BAL was only significantly higher in patients with progressing sarcoidosis and radiologically documented interstitial lung involvement; chitotriosidase activity in serum and BAL only increased in stage II–III sarcoidosis and was correlated with the extent and degree of lung damage.[30] However, the increased level of chitotriosidase in BAL of IPF patients is difficult to explain since chitotriosidase levels were not elevated in BAL of SSc patients. Different immunological mechanisms may be involved in lung damage in SSc than in IPF patients. For example, macrophages are activated by different stimuli and did not release chitotriosidase in BAL of these patients. A similar result suggesting that chitotriosidase was not a generic marker, elevated in all conditions characterised by macrophage activation, was normal activities of the enzyme in tuberculosis.[31] This showed that

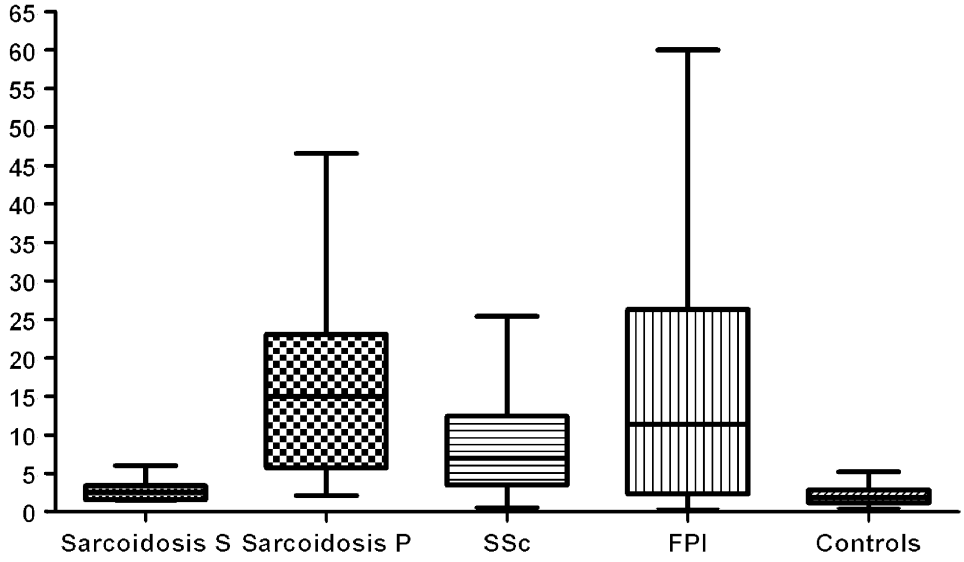


**Figure 2** Chitotriosidase activity (nmol/h/mg) in bronchoalveolar lavage of patients with stable sarcoidosis, progressing sarcoidosis, idiopathic pulmonary fibrosis, pulmonary fibrosis associated with systemic sclerosis and controls. The first line represents the minimum value, the second line is the 25° percentile, the third line is the mediane, the fourth line is the 75° percentile and the fifth line is the maximum value.

chitotriosidase is not elevated as would be expected in all granulomatous lung diseases with enhanced macrophage activation, possibly because other pathogenetic mechanisms activate different macrophage subsets which do not release chitotriosidase. The lack of increase in chitotriosidase recorded in SSc patients may also be due to the limited number of patients enrolled in the study. A future task will be to increase the population of SSc patients in order to confirm this result. As with other markers of sarcoidosis,[6] alveolar macrophages involved in inflammatory/fibrotic processes at alveolar level may have similar activation patterns in sarcoidosis and IPF (different from that in SSc), leading to enhanced expression of chitotriosidase activity in both diseases. This enzyme activity may therefore be considered a specific expression of enhanced macrophage activation during lung fibrogenesis and not an aspecific marker that increases in all pathological conditions characterised by macrophage activation. The recent finding that chitinases contribute to T-helper-2 inflammation in humans,[38] through an IL-3-dependent mechanism, could explain chitotriosidase increase at alveolar level in IPF patients in whom evolution towards chronic disease could involve type-2 cytokines and mediators.[39]

Malaguarnera et al.[20,21] report an increase in serum levels of chitinase-1 mRNA after macrophage stimulation with TNFalpha, IFNgamma and LPS and suggested that this enzyme may play a role in immune response. They report that prolactin promoted chitotriosidase gene expression in human macrophages, while interleukin-10 stimulation down-regulated chitotriosidase synthesis.[21,40] Different subpopulations of macrophages activated by distinct cytokines may be involved in the pathogenesis of ILD. They seem to be characterised by expression of mediators such as CCL18 (and perhaps chitotriosidase) and could play a role in the induction of collagen synthesis by fibroblasts.[6,41,42]

The increase in chitotriosidase activity in sarcoidosis patients both at alveolar and peripheral levels and in IPF patients only in BAL could be due to the fact that sarcoidosis is a multisystemic disorder with generally altered immune responses,[5,43,44] while IPF is an interstitial disorder, limited to the lungs, with immunological alterations only at alveolar level.[2,45–47]

Further studies with a greater number of patients are needed to define the role of chitotriosidase in the prognosis of sarcoidosis severity and its involvement in the pathogenesis of ILD.

## Acknowledgements

We thank A. Collodoro for performing bronchoalveolar lavage and R.M. Refini and M.G. Pieroni for lung function tests. This research was supported by funds (PAR) from the University of Siena.

## References

1. American Thoracic Society (ATS), European Respiratory Society (ERS). Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. *Am J Respir Crit Care Med* 2000;**161**:646–64.
2. American Thoracic Society (ATS), European Respiratory Society (ERS). International multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2002;**165**:227–304.
3. Selman M, King T, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med* 2001;**134**:136–51.
4. Rottoli P, Bargagli E. Is bronchoalveolar lavage obsolete in the diagnosis of interstitial lung disease? *Curr Opin Pulm Med* 2003;**9**:418–25.
5. Hunninghake GW, Costabel U, Ando M, et al. ATS/ERS/WASOG statement on sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1999;**16**:149–73.
6. Prasse A, Pechkovsky DV, Toews GB, et al. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am J Respir Crit Care Med* 2006;**173**: 781–92.
7. Antoniou KM, Tsiligianni I, Kyriakou D, et al. Perforin down-regulation and adhesion molecule activation in pulmonary sarcoidosis: an induced sputum and BAL study. *Chest* 2006;**129**:1592–8.
8. Ziegenhagen MW, Rothe ME, Schlaak M, Muller-Quernheim. BAL and serologial parameters reflecting the severity of sarcoidosis. *Eur Respir J* 2003;**21**:407–13.
9. Drent M, Jacobs JA, de Vries J, et al. Does the cellular BAL fluid profile reflect the severity of sarcoidosis? *Eur Respir J* 1999;**13**:1338–44.
10. Drent M, De Vries J, Lenters M, Lamers RJ, et al. Sarcoidosis: assessment of disease severity using HRCT. *Eur Radiol* 2003; **13**:2462–71.
11. Guerrieri C, Bortoli M, Brunetta E, Piazza F, Agostani C. Cytokines, chemokines and other biomolecular markers in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;**22S**:9–14.
12. Renkema GH, Boot RG, Au FL, et al. Chitotriosidase, a chitinase, and the 39 kDa human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *Eur J Biochem* 1998;**251**:504–9.
13. Altarescu G, Rudensky B, Abrahamov A, et al. Plasma chitotriosidase activity in patients with beta-thalassemia. *Am J Hematol* 2003;**72**:285–6.
14. Aguilera B, Ghauharali-van der Vlugt K, Helmond MTJ, et al. Transglycosidase activity of chitotriosidase. *J Biol Chem* 2003;**42**:40911–6.
15. Wajner A, Michelin K, Burin MG, et al. Biochemical characterisation of chitotriosidase enzyme. Comparison between normal individuals and patients with Gaucher and Niemann–Pick diseases. *Clin Biochem* 2004;**37**:893–7.
16. Van Eijk M, van Roomen CP, Renkema GH, et al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. *Int Immunol* 2005;**15**:1505–12.
17. Labadaridis I, Dimitriou E, Theodorakis M, et al. Chitotriosidase in neonates with fungal and bacterial infections. *Arch Dis Child Fetal Neonatal Ed* 2005;**90**:531–2.
18. Casal JA, Robles A, Tutor JC. Serum markers of monocyte/macrophage activation in patients with Alzheimer's disease and other types of dementia. *Clin Biochem* 2003;**36**:553–6.
19. Korolenko TA, Zhanaeva SY, Falameeva OV, et al. Chitotriosidase as a marker of macrophage stimulation. *Bull Exp Biol Med* 2000;**130**:948–50.
20. Malaguarnera L, Musumeci M, Di Rosa M, Scuto A, Musumeci S. IFN gamma, TNF-alpha and LPS promote chitotriosidase gene expression in human macrophages. *J Clin Lab Anal* 2005; **19**:128–32.
21. Di Rosa M, Musumeci M, Scuto A, Musumeci S, Malaguarnera L. Effect of interferon-gamma, interleukin-10, lipopolysaccharide and tumor necrosis factor alpha on chitotriosidase synthesis in human macrophages. *Clin Chem Lab Med* 2005; **43**:499–502.

22. Bouzas L, Carlos Guinarte J, Carlos Tutor J. Chitotriosidase activity in plasma and mononuclear and polymorphonuclear leukocyte populations. *J Clin Lab Anal* 2003;**7**:271–5.
23. Quintana L, Monasterio A, Escuredo K, et al. Identification of chitotriosidase isoforms in plasma of Gaucher disease patients by two-dimensional gel electrophoresis. *Biochim Biophys Acta* 2006;**1764**:1292–8.
24. Vedder AC, Cox-Brinkman J, Hollak CE, et al. Plasma chitotriosidase in male Fabry patients: a marker for monitoring lipid-laden macrophages and their correction by enzyme replacement therapy. *Mol Genet Metab* 2006;**89**:239–44.
25. Michelakakis H, Dimitriou E, Labadaridis I. The expanding spectrum of disorders with elevated plasma chitotriosidase activity: an update. *J Inherit Metab Dis* 2004;**27**:705–6.
26. Wajner A, Michelin K, Burin MG, et al. Biochemical characterisation of chitotriosidase enzyme. Comparison between normal individuals and patients with Gaucher and with Niemann–Pick diseases. *Clin Biochem* 2004;**37**:893–7.
27. Artieda M, Cennaro A, Ganan A, et al. Serum chitotriosidase in subjects with atherosclerosclerosis. *Arterioscler Thromb Vasc Biol* 2003;**23**:1645–52.
28. Barone R, Simpore J, Malaguarnera L, Pignatelli S, Musumeci S. Plasma chitotriosidase activity in acute *Plasmodium falciparum* malaria. *Clin Chim Acta* 2003;**331**:79–85.
29. Grosso S, Margollicci MA, Bargagli E, et al. Serum levels of chitotriosidase as a marker of disease activity and clinical stage in sarcoidosis. *Scand J Clin Lab Invest* 2004;**64**:57–62.
30. Bargagli E, Margollicci M, Perrone M, et al. Chitotriosidase levels in BAL of patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2006, in press.
31. Bargagli E, Margollicci MA, Nikiforakis N, et al. Chitotriosidase levels in serum of patients with sarcoidosis and pulmonary tuberculosis. *Respiration* 3 March 2007 [Epub ahead of print].
32. Rottoli P, Magi B, Perari MG, et al. Cytokine profile and proteome analysis in BAL of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. *Proteomics* 2005;**5**:1423–30.
33. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–54.
34. Calhoun WJ, Salisbury SM, Chosy LW, Busse WW. Increased alveolar macrophage chemiluminescence and airspace cell superoxide production in active pulmonary sarcoidosis. *J Lab Clin Med* 1988;**112**:147–56.
35. Papakosta D, Kyriazis G, Gioulekas D, et al. Variations in alveolar cell populations, lymphocyte subsets and NK cells in different stages of active pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2005;**22**:21–6.
36. Malaguarnera L, Rosa MD, Zambito AM, et al. Potential role of chitotriosidase gene in nonalcoholic fatty liver disease evolution. *Am J Gastroenterol* 2006;**101**:2060–9.
37. Malaguarnera L, Di Rosa M, Zambito AM, et al. Chitotriosidase gene expression in Kuppfer cells from patients with nonalcoholic fatty liver disease. *Gut* 2006;**55**:1313–20.
38. Elias JA, Homer RJ, Hamid Q, Lee CG. Chitinases and chitinase like proteins in T(*H*)2 inflammation and asthma. *J Allergy Clin Immunol* 2005;**116**:497–500.
39. Agostini C, Semenzato G. Cytokines in sarcoidosis. *Semin Respir Infect* 1998;**13**:184–96.
40. Malaguarnera L, Musumeci M, Licata F, et al. Prolactin induces chitotriosidase gene expression in human monocyte-derived macrophages. *Immunol Lett* 2004;**94**:57–63.
41. Song E, Ouyang N, Horbelt M, et al. Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. *Cell Immunol* 2000;**204**:19–28.
42. Atamas SP, Luzina IG, Choi J, et al. Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts. *Am J Respir Cell Mol Biol* 2003;**29**:743–7.
43. Drent M, Costabel U. Sarcoidosis. *Eur Respir Monogr* 2005; **10**(32):13.
44. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. *Lancet* 2003;**361**:1111–8.
45. Selman M, Pardo A. Idiopathic pulmonary fibrosis: an epithelial/fibroblastic cross-talk disorder. *Respir Res* 2002;**3**:3.
46. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. *N Engl J Med* 2001;**345**:517–25.
47. Strieter RM. Pathogenesis and natural history of usual interstitial pneumonia. The whole story or the last charter of a long novel. *Chest* 2005;**128**:526S–32S.