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Bronchoalveolar lavage chitotriosidase activity as a biomarker of sarcoidosis

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Abstract. *Background:* Chitotriosidase (CTO) was shown to be a good biomarker of sarcoidosis. Increased levels in bronchoalveolar lavage fluid (BALF) were reported and associated with more severe forms of the disease. *Objectives:* The aim of the study was to evaluate the value of CTO in BALF as a routine biomarker of sarcoidosis. *Methods:* The study included 85 patients in 9 control subjects in whom serum and BALF CTO were measured. *Results:* Significantly higher CTO levels were detected in BALF of sarcoidosis patients than in control subjects ($p < 0.001$). There was good correlation between serum and BALF CTO levels in sarcoidosis patients (Spearman's Rho 0.481, $p < 0.001$). Serum but not BALF CTO had good correlation with clinical parameters. Only in a group of patients with BALF CTO above upper normal range there was association of BALF CTO with impaired FVC ($p = 0.020$) and chest radiograph score (0-2 vs. 3-4, $p = 0.016$). *Conclusions:* In comparison to serum CTO no additional benefit of determining CTO in BAL for routine sarcoidosis work-up was shown. *(Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 313-317)*

Key words: sarcoidosis, chitotriosidase, BAL

Introduction

Chitotriosidase (CTO) is a human chitinolytic enzyme secreted by activated macrophages and polymorphonuclear neutrophils (1). Serum CTO is increased in variety of diseases, such as Gaucher's disease, beta-thalassemia, Plasmodium falciparum malaria and likely other diseases involving chronically activated macrophages (2). It is also increased in about 90% of patients with active sarcoidosis (3-6) which makes it a useful follow-up biomarker. Sarcoidosis is characterized by granuloma infiltration affecting mediastinal lymph nodes and lung parenchyma in most cases but almost any organ can be involved (7). CTO is thought to derive from sarcoid macrophages (5). It correlates with changes of chest radiographs and lung function (8). Additionally, it may have prognostic role, as the highest values were observed in patients with prolonged chronic sarcoidosis (6, 8).

To date, three studies addressed the value of determining CTO in bronchoalveolar lavage fluid (BALF) (5, 9, 10). BALF CTO was higher in progressive sarcoidosis compared to the stable sarcoidosis or control group. BALF CTO was higher in stage II and III patients compared to the stage 0 and I and higher in patients with more severe lung parenchyma

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involvement as evaluated by quantitative HRCT visual score. Moreover, the correlation between BALF CTO and angiotensin convertase enzyme (ACE) in serum was described (9). In patients with idiopathic pulmonary fibrosis or pulmonary fibrosis associated with systemic sclerosis BALF CTO was increased to similar extent as in progressive sarcoidosis patients, even though only modest increase of CTO could be observed in serum (10). Finally, by comparing plasma with BALF levels corrected for epithelial lining fluid volume it was shown that CTO in BALF derives not only from plasma but is also produced locally by alveolar macrophages. This was further confirmed by visualizing chitotriosidase cDNA in BALF macrophages with *in situ* hybridization (5). Nevertheless, high serum CTO values have also been associated with worse outcome of sarcoidosis similarly to BALF values (6, 8). It is thus unclear whether determining CTO in BALF adds value to the routine sarcoidosis evaluation.

The aim of the following study was to evaluate the value of CTO in BALF as a routine biomarker of sarcoidosis.

Methods

Subjects

The study comprised 88 patients with newly diagnosed sarcoidosis according to ATS/ERS/WASOG criteria (11) at the Department of pulmonary diseases of University medical centre Ljubljana, Slovenia. Patients were prospectively included from April 2007 to July 2013 immediately after the first diagnostic work-up. At the time of BALF sampling all patients were corticosteroid naïve. In three subjects there was no CTO activity (measured in serum) and they were excluded from further analysis (presumed to be homozygotes for CHIT1 duplication polymorphism). The data from 85 patients is reported in the results. The control group consisted of 9 healthy volunteers in whom bronchoscopy was performed for study purpose only. All of control subjects had measurable CTO activity in serum.

All subjects gave a written consent to participate in the study which was approved by the National Ethics Committee of the Republic of Slovenia.

Blood sampling

Venous blood was drawn into a tube without anticoagulant, centrifugated and immediately stored at -20°C.

Bronchoscopy and BAL

Bronchoscopy was made using a flexible bronchoscope which was inserted through the nose after local anaesthesia with 6-9 mL of 2% xylocaine. For CTO analysis BAL was made in the anterior segment of right upper lobe using 20 mL of sterile 0.9% NaCl. This was done before biopsies. The sample was then immediately centrifugated and stored at -20°C. This method was chosen so that the sampling did not interfere with other routine procedures.

Measurement of CTO activity

The CTO activity was determined in the serum and BALF using the 22 μM 4-methylumbelliferyl-β-D-N,N',N"-triacetylchitotriosiose (4 MU-chitotrioside, Sigma Chemical Co.) in citrate phosphate buffer (pH 5.2) as an enzymatical substrate. Five microlitres of serum or BALF was incubated with 100 μL of substrate for 1h at 37°C. The reaction was stopped by adding 2.5 mL of 0.3M glycine/NaOH buffer (pH 10.6). The reaction product, fluorescent 4-methylumbelliferone, was measured using a Perkin-Elmer fluorimeter at excitation wave lenght 365 nm and emission 465 nm. The CTO activity was expressed in nmol/mL/h.

Clinical assessment

The patients presenting with acute clinical picture consisting of bihilar lymphadenopathy, erythema nodosum and arthalgia were considered to have Löfgren's syndrome. Screening for extrapulmonary sarcoidosis was done in all patients.

Chest radiographs were evaluated by two chest radiologists who were unaware of the clinical data. In case of discrepancy in the evaluation, the reported result was reached by consensus. Firstly, the chest radiograph readings were classified into pulmonary sarcoidosis stages: stage 0 (normal chest radiograph), stage I (bilateral hilar lymphadenopathy), stage II (bilateral hilar lymphadenopathy accompanied by

parenchymal infiltration), stage III (parenchymal infiltration without hilar lymphadenopathy) and stage IV (advanced fibrosis with evidence of honeycombing, hilar retraction). Secondly, granuloma infiltration in pulmonary parenchyma was graded as described previously (12). The extension of infiltration was scored from 0 to 4: 0 for no infiltration, 1 for up to 25% of lung field involved, 2 for up to 50%, 3 for up to 75%, and 4 if the whole lung field was involved.

Forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLco) were measured using standard technique. DLco was measured using the single-breath method. FVC and DLco were classified as normal (≥80%) or as impaired (<80%).

Statistics

For calculations the statistical program SPSS 15.0 was used. The data did not have normal distribution so the non-parametric tests were used. The data were expressed as median and interquartile range (IQR). A p value <0.05 was considered as significant.

Results

The study and control group were matched in the gender but not in the age – the control subjects were significantly younger. Significantly higher levels of CTO were detected both in the serum and BALF of sarcoidosis patients compared to control subjects (Table 1). However, only 51 (60%) patients had increased CTO above the upper range of control group (3.4 nmol/mL/h).

There was a good correlation between serum and BALF CTO in sarcoidosis patients (Spearman's Rho 0.481, p < 0.001). The data are shown in Figure 1. The correlation between serum and BALF CTO did not reach significance in the control group (p = 0.09), possibly due to low number of control subjects.

CTO in serum was significantly lower in patients with Löfgren's syndrome than in patients with other clinical pictures (median [IQR], 420 [195] vs. 788 [676] nmol/mL/h, p = 0.008). CTO in serum was significantly higher in patients with impaired FVC (median [IQR], 624 [624] vs. 1392 [744]

Table 1. Characteristics of sarcoidosis patients and the control group. Patients with sarcoidosis had significantly higher serum and BALF CTO values

	Sarcoidosis n = 85	Control group n = 9	p
Gender	43 F (50.6%)	5 F (55.6%)	ns
Age in years median (range)	43 (22-70)	28 (22-38)	p < 0.001
Serum CTO (nmol/mL/h) median (IQR)	684 (672)	21 (17)	p < 0.001
BALF CTO (nmol/mL/h) median (IQR)	4.6 (7.5)	2.7 (1.0)	p < 0.001
Body mass index median (range)	27 (17-44)		
Smoking current smokers	8 (9.4%)		
Löfgren's syndrome	13 (15.3%)		
Extrapulmonary	23 (27.1%)		
FVC < 80%	12 (14.1%)		
DLco < 80%	27 (31.8%)		
Chest radiograph stage			
0	3 (3.5%)		
I	32 (37.6%)		
II	44 (51.8%)		
III	6 (7.1%)		
Chest radiograph score			
0	35 (41.2%)		
1	9 (10.6%)		
2	15 (17.6%)		
3	20 (23.5%)		
4	6 (7.1%)		

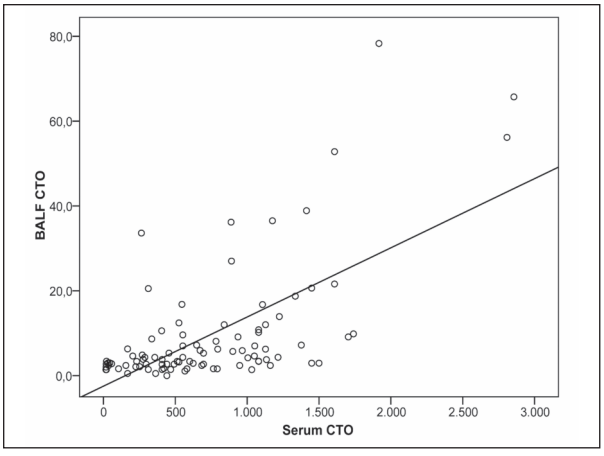

Fig. 1. Correlation between serum and BALF CTO in patients with sarcoidosis (n = 85, Spearman's Rho 0.481, p < 0.001). CTO values are in nmol/mL/h for serum and BALF. The line shows linear regression model (R-square 0.42)

nmol/mL/h, p = 0.005) or DLco (median [IQR], 552[591] vs. 1080 [450] nmol/mL/h, p = 0.001). There was no correlation between serum CTO and chest radiograph stages but serum CTO was higher in patients with chest radiograph scores 3 and 4 as compared to scores 0 to 2 (median [IQR], 581 [600] vs. 1080 [678] nmol/mL/h, p = 0.034). This is similar to already published data (8, 12). On the other hand, BALF CTO did not correlate with any clinical parameter (gender, age, BMI, smoking, Löfgren's syndrome, extrapulmonary sarcoidosis, lung function, chest radiograph stage or score). If analysis was repeated only on patients with BALF CTO above the upper normal limit, there were significantly higher CTO values in patients with impaired FVC compared to patients with normal FVC (median [IQR], 8.10 [9.06] vs. 19.7 [45.7] nmol/mL/h, p = 0.020) and in chest radiograph scores 3 and 4 compared to scores 0 to 2 (median [IQR], 7.10 [7.47] vs. 13.92 [44.7] nmol/mL/h, p = 0.016).

Discussion

In this study higher CTO levels were detected in the BALF of sarcoidosis patients as compared to control subjects. This is consistent with previously reported data (5, 9). There was a good correlation between serum and BALF CTO which was not previously reported but could be anticipated as correlation between BALF CTO and serum ACE was described (9). It was previously showed that alveolar macrophages produce CTO and that BALF values corrected for dilution exceed plasma levels (5). It is thus unlikely that the observed correlation is only a consequence of transudation of CTO from plasma to alveolar space. A more likely explanation is that alveolar and systemic macrophages are activated via similar pathways, such as tumor necrosis factor alpha (up-regulation) and interleukin-10 (down-regulation) (13). It is interesting that higher BALF beta-glucan levels and environmental fungal exposure were linked to lower serum interleukin-10 levels (14). Local antigen (chitin) exposure could possibly drive additional CTO secretion but as yet there are no data to support this hypothesis.

BALF CTO levels in the described group of patients did not correlate with any clinical parameter of sarcoidosis patients, including chest radiographs stage. It was previously reported that BALF CTO was higher in patients with chest radiographs stages II and III as compared to 0 and I (9). But in this study high proportion of patients (25/28) with stage II and III also had progressive sarcoidosis which is higher than expected for these stages (11). This could indicate a difference in the sarcoidosis patient population. Another possible explanation for this discrepancy is a known large interobserver variability in staging of chest radiographs (15). On the other hand, in a subgroup of patients with BALF CTO above normal range there were significantly higher values of BALF CTO in patients with impaired FVC and with chest radiographs scores 3 and 4 (compared to 0 – 2). This is consistent with previously published correlation between BALF CTO and the degree of lung infiltration evaluated by HRCT (9). Nevertheless, serum CTO levels were in better association with impairment of lung function and with chest radiograph score. This may be due to relatively large proportion of patients with negative (normal range) CTO in BALF likely caused by the methodological problems discussed in the next section.

The data collected in this study have to be interpreted in light of several shortcomings. First of all, the control group was not matched in age with sarcoidosis patients, which is a known to have a weak positive correlation with CTO (6, 16, 17). Secondly, BAL was obtained with relatively small amount of fluid, a mini-bronchoalveolar lavage like technique. Although this is not standardized technique it has been previously successfully used to detect various biomarkers (18, 19). Its advantage is that it is relatively non-invasive and could be easily used as an add-on to other examinations. Finally, no method for determining dilution of the sample was available (i.e. determination of epithelial lining fluid volume).

In summary, this study confirmed that CTO in BALF is increased in patients with sarcoidosis. Good correlation between serum and BALF CTO was showed. Only after exclusion of patients with values within normal range BALF CTO could be associated with chest radiograph score and lung function. Serum CTO correlated better with clinical presentation of sarcoidosis and we could show no additional benefit of determining CTO in BALF for routine sarcoidosis work-up.

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