EXHALED CARBON MONOXIDE IN SARCOIDOSIS

G. Ciarleglio, R.M. Refini, M.G. Pieroni, V.A.M. Martino, E. Bargagli, P. Rottoli, P. Sestini

Department of Clinical Medicine and Immunological Science, Section of Respiratory Diseases, University of Siena School of Medicine

ABSTRACT. *Background:* Exhaled Carbon monoxide has been proposed as a non-invasive marker in several inflammatory diseases of the lung, but no data are available in patients with sarcoidosis. *Methods:* We evaluated the levels of exhaled CO in 78 nonsmoker patients with sarcoidosis and we compared the results with 25 healthy non smoker controls, of 25 patients with a variety of interstitial lung diseases, and 77 smokers. *Results:* Mean value of exhaled CO in sarcoidosis was 3.3 (2.9-3.8) ppm (GM with 95% CI in parenthesis), resulting significantly higher than both normal controls, 1.4 (1.2-1.7) ppm (p<0.001), and clinical controls, 2.1 (1.7-2.7) ppm (p<0.02). All these levels, however, were markedly lower than those observed in smokers, 14.6 (12.7-16.9) ppm. No correlation was found with radiological stage, steroid therapy, respiratory function, or serum ACE activity. Using an upper normal value of 4 ppm, an increased level of exhaled CO was found in 50% of patients with sarcoidosis, in 24% of clinical controls, and in 97% of smokers. *Conclusions:* Our data indicate that significant release of endogenous CO occurs in sarcoidosis. It is unlikely that the measurement of exhaled CO could be of diagnostic usefulness, due to its low specificity and to the possible influence by occasional or passive smoke. *(Sarcoidosis Vasc Diffuse Lung Dis 2008; 25: 46-50)*

KEY WORDS: sarcoidosis, interstitial lung diseases, carbon monoxide, carboxyhemoglobin

INTRODUCTION

Carbon monoxide is a non irritant toxic gas produced by combustion of organic materials (1, 2). Measurement of CO in exhaled air has proven useful to detect exposure to exogenous sources of CO such as tobacco smoke, particularly in smoking cessation (3), and in fire fighters during fire attacks (4).

Carbon monoxide can also be endogenously produced by heme oxygenase enzymes (HO). HO enzymes catalyze heme degradation to biliverdin IXa, iron and CO. Subsequently, biliverdin IXa is converted to bilirubin IXa, which in turn is transformed in bile pigments with antioxidant properties (2.) There are three isoforms of heme oxygenase enzymes: HO1, HO2 and HO3. HO1 is highly inducible by several stimuli such as hypoxia, hyperoxia, cytokines and others associated with oxidative stress (2, 5) which contributes to pulmonary tissue damage in diffuse lung diseases (6).

Indeed, an increase in exhaled carbon monoxide (e-CO) has been observed in patients with a variety of inflammatory lung diseases, including bronchiectasis (7), cystic fibrosis (8, 9), asthma and bronchial asthma exacerbation (10-13) pneumonia (14, 15) and interstitial pulmonary fibrosis (5, 15).

Sarcoidosis is a multisystemic disease of unknown ethiology, often involving lung lymphnodes and tissues, whose pathology is characterized by diffuse granulomatous inflammation. The clinical course of sarcoidosis is highly variable, but currently

there are no non-invasive methods for assessing disease activity. Interestingly, an increase in the expression of HO1 has been reported in alveolar macrophages of patients with sarcoidosis (16). However, no data is available on the levels of exhaled CO in pulmonary sarcoidosis.

To evaluate the possible use of exhaled CO as a non-invasive marker of sarcoidosis, we compared the levels of exhaled CO of a group of patients with sarcoidosis, a group of patients with other interstitial lung diseases (mostly idiopathic pulmonary fibrosis) a group of healthy non smoker controls, and a group of smokers.

Patients and methods

We studied 78 patients with sarcoidosis (25 males 53 females, age 52±13 years, M±SD) attending the sarcoidosis Clinic of our institution (Tab. 1). The diagnosis and staging of sarcoidosis was established using internationally agreed criteria (17). At least one marker of clinical activity (as defined in 17) was present in 47 patients, and clinical progression (worsening radiography or lung function) in 26 (Tab. 2). Steroid therapy was reported by 33 patients (44%), at a mean daily dose of 12±7 mg prednisone. Radiological stage was 0 in 18%, I in 24%. II in 26% and III in 32% of patients with sarcoidosis, without significant differences between those with or without at least one marker of clinical activity.

This group was compared with two groups of controls: a group of 25 healthy subjects (7 males and 18 females, mean age 39±10 years), and a group of clinical controls consisting of 25 patients with different interstitial lung diseases: 12 idiopathic pulmonary fibrosis, 2 systemic sclerosis, 2 bronchiolitis, 2 eosinophilic pneumonitis, 2 primary pulmonary

hypertension, and one each with asbestosis, silicosis, Sjogren syndrome, Churg Strauss disease, Histiocytosis X (7 males and 18 females, mean age 63±12 years). All the subjects were non-smoker for at least one year and had been free from viral infections or other unrelated inflammatory diseases in the previous 4 weeks. As a comparison group, we also used data from 77 smokers attending for the first time our smoking cessation clinic. They were 55% males, mean age was 47±1 years (M± S), and mean Fagerstrom score was 5.9±0.2.

Each patient in the sarcoidosis and in the clinical control group underwent, on the same day, determination of exhaled CO, pulmonary function tests and DLCO. Exhaled carbon monoxide was measured before measuring DLCO using a Bedfont micro Smokerlyzer (http://www.smokerlyzer.com) with a detection limit of 1 ppm of CO. The patients were instructed to take a maximal inspiration from functional residual capacity to total lung capacity, to hold the breath for 15", and then to exhale gently trough the mouthpiece of the Smokerlyzer. Exhaled CO values were expressed both in ppm and as a percentage of carboxyhemoglobin (COHb) over total Hb. Spirometry, body plethysmography and DLCO were measured using a Masterscreen Body plethysmograph (Jaeger Toennies, Germany).

Statistical analysis. Differences between groups were investigated by two-ways or one-way ANOVA followed by Scheffé test to adjust for comparisons among multiple groups. A logarithmic transformation was applied to data of exhaled CO to reduce heterogeneity of the variances. Unless stated otherwise, data are presented as the geometric mean (GM) with 95% CI in parenthesis. All the analyses were performed using the statistical package Stata 8 on a PC-compatible personal computer (Stata Corporation, College Station, Tx, USA)

Table 1. Characteristics of the study groups.

	N	Female Sex	Age	Active°	%VC	%FEV1	%TLC	%RV	%DLCO
Sarcoidosis	78	53	52±1*	47	103±2§	97±2	100±2	102±3	83±2
Stage 0	14	11	53±3	7	115±6	106±6	108±3	110±5	89±5
Stage 1	19	12	50±3	12	104±3	102±3	104±2	109±4	91±3
Stage 2	20	13	51±3	14	104±3	98±3	100±3	97±5	83±4
Stage 3	25	17	54±3	14	93±4	89±5	94±4	96±6	73±5
Clinical Controls	25	18	63±2	-	79±4	76±5	83±5	87±7	59±5
Healthy Controls	25	17	39±2	-	-	-	-	-	-

*M ±SE
§ Percent predicted, M ±SE
° At least one marker of activity, see table I

Table 2. Parameters of clinical activity.

Parameter of activity (17)	N
Serum ACE > 55 U/min·l	22
Fever	1
Symptoms	10
BAL CD4/CD8 >2,5*	2
Hypercalcemia	4
Uveitis	2
Erythema Nodosum	2
Polyarthralgia	4
Lymphoadenopathy	8
X-Ray progression	20
Worsening respiratory function	7
Any of the above	47
X-ray progression or worsening resp. function	26

*Recent BAL available for only 3 of the patients

RESULTS

Mean levels of exhaled CO in nonsmoking controls (Fig. 1) resulted 1.4 (1.3-1.7) ppm (GM with 95% CI in parenthesis). In active sarcoidosis, mean level was 3.3 (2.9-3.8) ppm, in inactive sarcoidosis 2.7 (2.1-3.3) ppm, and in clinical controls resulted 2.1 (1.7-2.7) ppm. Statistical analysis by two-ways anova indicated a significant difference between the groups (p<0.005) without significant differences due to age or sex. Analysis by one-way ANOVA followed
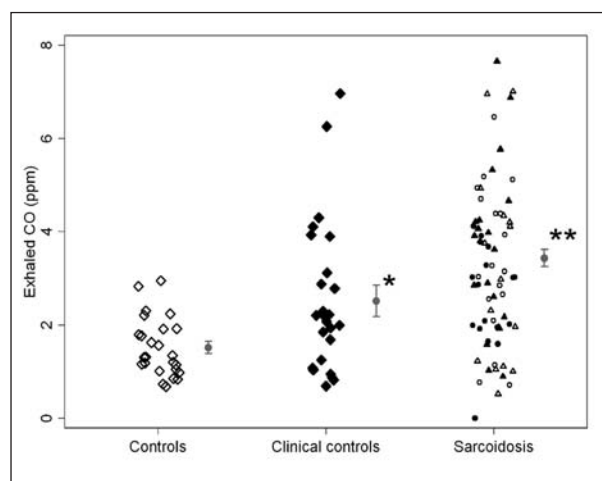

Fig. 1. Exhaled CO in 25 healthy controls (open squares), 25 clinical controls with miscellaneous interstitial lung diseases (closed squares), and 58 patients with sarcoidosis (open circles: stages 0-I, no therapy; closed circles: stages 0-I, steroid therapy; open triangles: stages II-III, no therapy; closed triangles: stages II-III, steroid therapy). Error bars represent the mean with SE.
* p<0.05 vs healthy controls. ** p<0.001 vs healthy controls, p<0.02 vs clinical controls.

by Scheffe' test indicated a significant difference between controls and all the other groups (active sarcoidosis p<0.001, inactive sarcoidosis p<0.001, and clinical controls p<0.05) and also between active sarcoidosis and clinical controls (p<0.01). The highest values of exhaled CO in clinical controls were observed in one patient with silicosis and one with Langerhans cell histiocytosis (Fig. 1).

In multivariable ANOVA models, no significant differences were found among patients with sarcoidosis according to clinical activity, progression, radiological stage, or steroid therapy. A significant difference was observed according to gender: men 3.7 (1.1) ppm, females 2.4 (1.1) ppm, adjusted difference 1.4 (1.1) ppm, p<0.01.

No significant correlations were observed between the level of exhaled CO and respiratory function (VC, IC, FEV1, TLC, RV, DLCO), or serum levels of angiotensin converting enzyme.

The levels of exhaled CO in sarcoidosis and in other interstitial lung diseases, however, were markedly lower than those obtained in the group of smokers, where it resulted 14.6 (12.7-16.9) ppm. By comparison, the maximum value of exhaled CO observed in a patient with sarcoidosis was 8 ppm, with all the other patients presenting values of 7 ppm or less. The value of exhaled CO corresponding to the GM minus 2 SD in the group of smokers was 4.16 ppm, while the value corresponding to the GM plus 2 SD in nonsmoking controls was 3.14 ppm. Accordingly, we chose a threshold of 4.0 ppm as a practical normal limit for nonsmokers. A value of exhaled CO equal or higher of this limit was observed in 50% of the patients with sarcoidosis (55% of those with at least one marker of clinical activity 42% among the ones on therapy, NS), in 24% of clinical controls (p <0.02 vs sarcoidosis by chi square test), in none of the nonsmoking controls, and in 96% of the smokers.

DISCUSSION

We showed that the levels of exhaled CO are increased in patients with sarcoidosis as compared to both normal controls and to patients with other miscellaneous interstitial lung diseases. We have not investigated the origin of this exogenous CO, but it seems likely that this is the result of HO1 activation

due to oxidative stress (2, 5), since increased immunoreactivity for HO1 has been observed in alveolar macrophages and in granulumas in patients with sarcoidosis (16). This interpretation would also be in agreement with the results obtained in a variety of other pulmonary diseases, such as asthma, allergic rhinitis, Cystic fibrosis, bronchiectasis and pneumonia (7, 18-20).

Unlike what was reported in asthma (24), we did not detect differences between patients treated or not with steroids. However, the intensity of the inflammatory damage and the response to steroids is much more variable in sarcoidosis than in asthma, and a prospective study would be needed to properly evaluate the effect of steroids and the correlation with clinical and radiological markers of activity of the disease. Indeed, no correlation with stage, steroid therapy, or disease activity was observed in sarcoidosis also with levels of exhaled NO, another proposed non-invasive marker of inflammation (25). Similarly, it is not surprising that we did not observe any correlation between the level of exhaled CO and the extent of lung involvement as evaluated by radiological and functional parameters. It is well known that radiological stage and respiratory function parameters are poorly correlated with the intensity of the inflammatory process in sarcoidosis (26). Furthermore, exhaled CO may reflect CO produced not only in the lung, but also in other organs (27, 28), and multisistemic involvement is common in sarcoidosis. Therefore, the level of exhaled CO would reflect the response to oxidative damage due to the total burden of granulomatous inflammation in the body rather than just the extent of lung disease. Although it could be possible to estimate the local or distant production of CO by comparing measurements in venous and arterial blood (14), this was not done in this preliminary work.

The consequences of this increased production of CO in sarcoidosis are unclear. It is possible that it represents a protective response to local inflammation, since CO has been shown to be able to suppress the production of proinflammatory cytokines such as TNF alpha and Il-1B, and inducing the anti-inflammatory cytokine Il-10 (21). Furthermore, gene therapy with HO1 cDNA (22) or exposure to low CO concentrations (23) have been recently shown to inhibit fibroblast proliferation and to exert a protective activity against the development of bleomycin-induced fibrosis in mice. However, further studies would be necessary to address these hypotheses.

Overall, our data indicate that the inflammatory process of sarcoidosis is associated to an increased levels of CO, possibly as a consequence of oxidative damage. While this information is useful for the knowledge of the pathogenetic mechanisms of the disease, it seems unlikely that it would be of diagnostic usefulness. Firstly, although the mean levels of exhaled CO are statistically increased in the group of patients with sarcoidosis, there is considerable overlap with results obtained in healthy and clinical controls. Indeed, only one patient had a value of CO slightly higher than 7 ppm, which is considered the upper normal value in nonsmokers in smoking cessation (3). Even after selecting a less conservative threshold based on our own data, we found that only about 50% of the patients with sarcoidosis had an increased level of exhaled CO, and that specificity evaluated in a group of patients with diseases which could be included in the differential diagnosis with sarcoidosis was only about 75%. Although sensitivity in this case-control could be underestimated because of the presence of patients in therapy or with inactive disease, and could result somewhat higher in a properly conducted diagnostic study performed on incident cases in conditions of diagnostic uncertainty, it is unlikely that specificity would increase. Furthermore, such a low threshold of positivity would be very sensitive to errors due to occasional or even passive smoking (3). Nevertheless, our data suggests that in practical use of exhaled CO to assess smoking abstinence in smoking cessation programs, an higher threshold should be used in patient with sarcoidosis and other lung diseases compared to healthy subjects. Furthermore, since the prognostic value of a test cannot be evaluated in a case-control study, a prospective follow-up study would be useful to monitor the changes on exhaled CO over time and to evaluate the prognostic value of this measurement in patients with sarcoidosis.

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