Carbohydrate antigen 15-3 as a marker of disease severity in patients with chronic hypersensitivity pneumonitis*

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ABSTRACT

Objective: Biomarkers associated with mucin 1, such as Krebs von den Lungen-6 and carbohydrate antigen (CA) 15-3, are increased in various interstitial lung diseases. Our aim was to determine whether CA 15-3 could be considered a biomarker of disease severity in patients with chronic hypersensitivity pneumonitis (cHP). **Methods:** This was a prospective observational study involving adult patients with cHP. Serum levels of CA 15-3 were measured and were correlated with variables related to disease severity and extension. HRCT scans were quantitatively analyzed using a computational platform and an image analysis tool (Computer Aided Lung Informatics for Pathology Evaluation and Rating). CA 15-3 levels were normalized by logarithmic transformation. **Results:** The sample comprised 41 patients. The mean age of the patients was 60.1 ± 11.6 years. The mean FVC in % of predicted was 70.3% ± 17.3%, and the median of the serum level of CA 15-3 was 48.1 U/mL. CA 15-3 levels inversely correlated with FVC in % of predicted (r = −0,30; p = 0,05), DLCO in % of predicted (r = −0,54; p < 0,01), and SpO_2 at the end of a 4-min step test (r = −0,59; p < 0,01), but they directly correlated with total quantitative HRCT scores (r = 0,47; p = 0,004), especially regarding ground-glass opacities (r = 0.58; p < 0,001). **Conclusions:** CA 15-3 is likely to be a biomarker of disease severity of patients with cHP, particularly regarding gas exchange abnormalities.

Keywords: Antigens, tumor-associated, carbohydrate; Alveolitis, extrinsic allergic; Biomarkers; Lung diseases, interstitial.

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an interstitial lung disease (ILD) caused by inhaled, mostly organic, antigens. The current classification differentiates HP between fibrotic and nonfibrotic HP.[1,2] The diagnosis of chronic HP (cHP) has prognostic and therapeutic implications.[2,3]

A biomarker able to detect disease activity and severity can be useful in cHP. Krebs von den Lungen-6 (KL-6) is a mucin 1 (MUC1) epitope. KL-6 is a membrane glycoprotein encoded by the *MUC1* gene and expressed on the surface of lung epithelial cells.[4] KL-6 is an important biomarker of various ILDs; however, KL-6 quantification assays are unavailable in most countries.[5] Carbohydrate antigen (CA) 15-3 is also a MUC1 epitope but, unlike KL-6, CA 15-3 quantification assays are widely available. CA 15-3 levels are correlated with KL-6 in ILDs, including a subset group of patients with HP.[6-8] In addition, CA 15-3 levels are correlated with disease extent on HRCT in patients with ILD associated with systemic sclerosis.[9]

The objective of the present study was to determine the role of CA 15-3 as a biomarker of disease severity in patients with cHP. We evaluated whether CA 15-3 levels would correlate with disease extent on the basis of perception of dyspnea, lung function, SpO_2 after exercising, and HRCT.

METHODS

This was a prospective observational study involving consecutive patients with cHP who sought medical attention at a university hospital located in the city of São Paulo, Brazil, between December of 2015 and October of 2017.

All patients underwent spirometry and DLCO measurement, and the results were compared with reference values.[10,11] SpO_2 was measured at rest and at the end of a 4-min step test (SpO_2-Ex).[12] Transthoracic echocardiography and HRCT results, as well as serum levels of CA 15-3 (Elecsys CA 15-3; Roche Diagnostics, Rotkreuz, Switzerland) and perception of dyspnea, measured by the modified Medical Research Council (mMRC) scale and the Mahler scale, were recorded.[13,14] The reference range of serum CA 15-3 using Elecsys CA 15-3 assay is < 26.4 U/mL.[15]

The inclusion criteria were as follows: being ≥ 18 years of age and being diagnosed with cHP in accordance with the criteria proposed by Salisbury et al.[2] for probable HP. Axial disease distribution on HRCT was added as a suggestive HRCT finding.[16] HP was classified as chronic in the presence of symptoms or radiological evidence of disease for at least 3 months. HP was classified as fibrotic or nonfibrotic according to the presence of findings indicative of fibrosis on HRCT.[1] Bronchoscopy, bronchoalveolar lavage, transbronchial lung biopsy (TBB), and/or surgical lung biopsy (SLB) were performed, taking contraindications into account, if definitive diagnosis was not reached by the abovementioned methods.[17]

The exclusion criteria were as follows: being a current smoker; having other potential causes for ILDs; having SpO_2 at rest < 89%; being unable to perform spirometry and DLCO measurement; having breast cancer, colon cancer, pancreatic disease, hepatitis, liver cirrhosis, or symptoms of gastroesophageal reflux disease; and having a change in diagnosis during follow-up.

We classified HP as active or inactive. HP was considered active if there was worsening of dyspnea or a significant decrease in FVC (≥ 10% in relation to baseline values) or in DLCO (≥ 15% in relation to baseline values) within the last 6-12 months prior to inclusion in the study. Improvement or stability in the perception of dyspnea and in lung function parameters indicated inactive HP.

All HRCT scans were acquired with 1-mm collimation, the findings being defined in accordance with the Fleischner Society recommendations.[18] Quantitative analysis of HRCT scans was performed using a computational platform (Lung Texture Analysis [LTA]; Imbio, Minneapolis, MN, USA) and an image analysis tool (Computer Aided Lung Informatics for Pathology Evaluation and Rating [CALIPER]; Imbio). LTA provides detailed quantification of textures by lung region and is able to identify accurately ILDs and other fibrotic conditions (such as honeycombing, reticular opacities, and ground-glass opacities) as well as hyperlucent areas, and normal lung parenchyma. A total score can be calculated.[19]

The present study was approved by the Research Ethics Committee of the *Universidade Federal de São Paulo* (Protocol no. 2.391.623). All patients gave written informed consent.

Statistical analysis

Continuous variables were expressed as means and standard deviations or medians and interquartile ranges. Categorical variables were described in absolute and relative frequencies.

Because CA 15-3 levels had a nonparametric distribution, the values were transformed into natural logarithms that adjusted to normal distribution (Shapiro-Wilk test). Then, the Pearson's correlation test was used to correlate serum levels of CA 15-3 with pulmonary function variables, and Spearman's correlation coefficients were used to correlate serum levels of CA 15-3 with Mahler scale scores and with HRCT scores derived from CALIPER. We used ANOVA followed by Tukey's test to compare serum levels of CA 15-3 with mMRC scale scores. All statistical analyses were performed with the IBM SPSS Statistics software package, version 21.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

The initial sample comprised 52 patients diagnosed with cHP: 9 and 2 were excluded prior to the beginning of the study and during the study period, respectively. Of those 2 patients, 1 presented with rapid progression of the disease, and the anatomopathological findings obtained from SLB revealed usual interstitial pneumonia, final diagnosis being idiopathic pulmonary fibrosis (IPF); and 1 was diagnosed with connective tissue disease during follow-up (Figure 1). Therefore, 41 patients were included in final analysis. The baseline characteristics of the final sample are shown in Table 1. At the time of initial evaluation, 29 patients (70.7%) had received no treatment.

All of the patients were exposed to inhaled antigens, mold and birds being the most frequent types of antigens, and presented with respiratory symptoms. Thirteen patients (31.7%) were exposed to both molds and birds. Of the 41 patients, in regard to their admission to the study, 22 (51.2%) and 19 (46.3%) had had a recent and a previous exposure to antigens, respectively.

In the sample as a whole, 13 (31.7%) and 28 (68.3%) of the patients, respectively, presented with one and with two or more tomographic findings suggestive of cHP. Features indicative of fibrosis on HRCT were present in 28 (68.3%) of the patients.

Of the 41 patients, 4 (9.8%) had a definitive diagnosis of cHP and needed to undergo no other diagnostic methods; 23 (56.1%) and 14 (34.1%) underwent TBB and SLB, respectively. Among those who underwent TBB, increased lymphocytes in bronchoalveolar lavage fluid (> 20%) were found in 15 patients.

Biopsies revealed findings that were diagnostic of or consistent with HP in 19 of the 37 patients (51%): classic HP, in 6 (2 by TBB and 4 by SLB); findings indicative of bronchiolar injury (bronchiolitis obliterans, peribronchiolar metaplasia, or air trapping), in 10 (3 by TBB and 7 by SLB); and airway-centered Interstitial fibrosis, in 3 (by SLB). Only 1 patient presented with a usual interstitial pneumonia pattern, but this pattern was associated with other typical HP findings.

Median serum levels of CA 15-3 were 48.1 U/mL, ranging from 13.2 U/mL to 228.7 U/mL. In the sample as a whole, 22 and 19 patients had inactive and active cHP, respectively, the mean natural logarithms of the serum levels of CA 15-3 being 3.65 ± 0.64 and. 4.20 ± 0.77 (t = 2.48, p = 0.02). Using the ROC curve and anti-normal logarithm transformation, the best cutoff point was 51.3 U/mL.
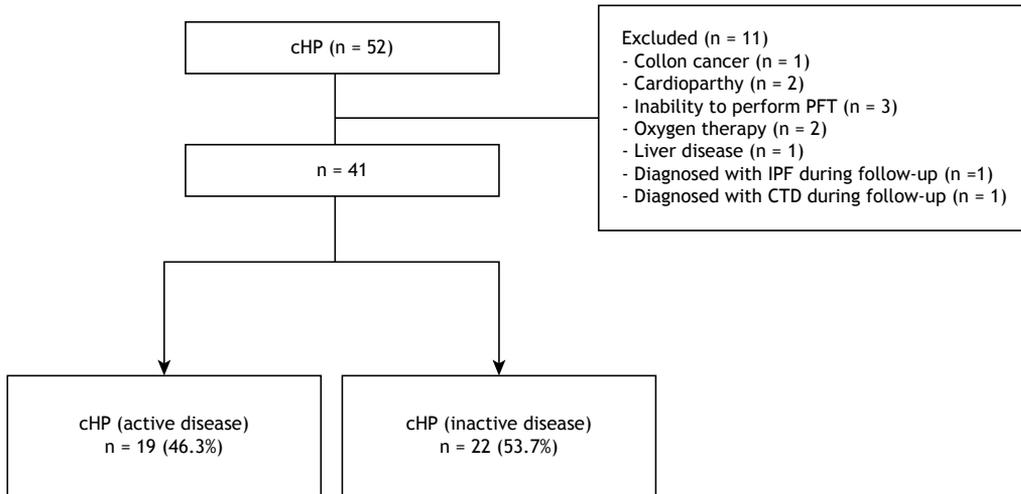
Figure 1. Flow chart of the study selection process. cHP: chronic hypersensitivity pneumonitis; PFT: pulmonary function test; IPF: idiopathic pulmonary fibrosis; and CTD: connective tissue disease.

Table 1. Baseline characteristics of the patients studied.[a]

Characteristic	(N = 41)
Age, years	60.1 ± 11.6
Female	30 (73.2)
Smoking status	
Nonsmoker	26 (63.4)
Former smoker	15 (36.6)
Lymphocytes in BALF, %	24 [4-76]
Lymphocytes in BALF > 20%[b]	13 (59)
FVC, % of predicted	70.3 ± 17.3
DLCO, % of predicted	54.5 ± 19.0
mMRC score	2 [1-3]
Mahler score	7 [5-8]
SpO_2 at rest	96 [93.5–97.0]
SpO_2-Ex	88 [83.5–92.5]
Serum CA 15-3, U/mL	48.1 [26.9-83.6]
HRCT findings	
Indicative of fibrosis	28 (68.3)
Ground-glass opacities	36 (87.8)
Centrilobular nodules	10 (24.4)
Honeycombing	11 (26.8)
Emphysema	13 (31.7)
Traction bronchiectasis	25 (60.9)
Bronchiolectasis	21 (51.2)
Air trapping	27 (65.8)
Axial distribution	24 (58.5)
Predominant disease in upper lobes	2 (4.9)
Pharmacological treatment at inclusion	
None	29 (71)
Prednisone	9 (22)
Immunosuppressant[c]	3 (7)

BALF: bronchoalveolar lavage fluid; mMRC: modified Medical Research Council; SpO_2-Ex: SpO_2 measured at the end of a 4-min step test; and CA: carbohydrate antigen. [a]Values expressed as n (%), mean ± SD, or median [IQR]. [b]n = 22. [c]Associated with prednisone in 2 patients.

Environmental exposure preceded the symptoms in all cases. No significant differences in CA 15-3 levels were found between the groups that had recent and previous environmental exposure (data not shown). Twenty-seven patients were able to avoid environmental exposure, which resulted in clinical improvement in 21 patients (but not in 6), 12 continued to be exposed, and that information was considered uncertain in 2. When we compared the patients who continued to be exposed to antigens plus those who avoided it but showed no clinical improvement (n = 18) with those who avoided environmental exposure and showed clinical improvement (n = 21), the CA 15-3 levels were lower in the latter group (4.241 ± 0.780 U/mL vs. 3.602 ± 0.634 U/mL; t = 2.82; p < 0.01). The ROC curve showed that the best cutoff point between the two groups was 55.3 U/mL.

No differences in CA 15-3 levels were found between nonsmokers and former smokers (data not shown). In addition, CA 15-3 levels were similar between patients who had had no treatment up to the time of initial evaluation (n = 29) and those who had been treated (n = 12): [3.97 ± 0.78 U/mL vs. 3.76 ± 0.68 U/mL; p = 0.40).

There was a statistically significant difference between CA 15-3 levels and mMRC scale scores (Z = 5.45; p < 0.01). Tukey's test showed that there was also a statistically significant difference between patients with an mMRC score = 3 and those with an mMRC score = 1-2 (p < 0.05, data not shown). There was a significant but poor inverse correlation between the perception of dyspnea measured by the Mahler scale and CA 15-3 levels ($r_s = -0.31$; p = 0.04). No differences were found regarding FVC in % of predicted (FVC%), DLCO in % of predicted (DLCO%), or SpO_2-Ex between nonsmokers and former smokers (data not shown).

There was a significant negative correlation between the serum levels of CA 15-3 and FVC% (r = −0.30; p

= 0.05), DLCO% (r= −0.54; p < 0.01), and SpO$_2$-Ex (r = −0.59; p < 0.01; Figure 2).

There were no significant differences in CA 15-3 levels between patients with (n = 28) and without (n = 13) findings indicative of fibrosis on HRCT (4.015 ± 0.711 U/mL vs. 3.681 ± 0.808 U/mL; p = 0.19).

By CALIPER analysis, there was a statistically significant correlation between CA 15-3 levels and the following HRCT scores: total ground-glass opacities, total honeycombing, total fibrosis score (i.e., total reticular opacities plus honeycombing), and total score (Table 2 and Figure 3). The correlation between serum levels of CA 15-3 and CALIPER quantification of total ground-glass opacities is shown in Figure 3.

DISCUSSION

The present study showed that, in patients with cHP, there is an inverse correlation of serum levels of CA 15-3 with FVC%, DLCO%, and SpO$_2$-Ex, as well as there is a direct correlation of those CA 15-3 levels with the extent of disease on HRCT, especially that related to the quantification of ground-glass opacities. There are various histopathological findings related to HP.[20-22] In the present study, we used the criteria proposed by Salisbury et al.[2] for the diagnosis of HP.

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention."[23] Several biomarkers have been studied in ILDs, especially in IPF, and can provide information about the course of the disease.[24,25] KL-6 is now classified as a human MUC1 protein. Regenerating type II pneumocytes are the primary cellular source of KL-6/MUC1 in the affected lungs of patients with ILD.[5] Extensive investigations performed primarily in Japan revealed that serum levels of KL-6/MUC1 are elevated in 70-100% of patients with various ILDs, including HP.[5] Sequential changes of serum levels of KL-6 can predict the progression of ILD.[26]

CA 15-3 is a tumor marker for many types of cancer, most notably breast cancer. Similarly to KL-6, it is also derived from MUC1, but CA 15-3 measurement is widely available, is fully automated, and has low costs. Previous studies showed that CA 15-3 levels have a high correlation with KL-6 levels in patients with ILDs, especially in those with fibrotic ILDs.[6-8] In one study, CA 15-3 levels were shown to be elevated in 26 patients with HP, and the correlation between KL-6 and CA 15-3 levels was very strong.[8] In another study involving patients with cHP, KL-6 levels were measured during different seasons and were shown to be increased during summer as a result of the greater humidity in the households at that time of the year.[27]

In our study, there was a statistically significant direct correlation between dyspnea scores and serum levels of CA 15-3. In addition, there was an inverse correlation between serum levels of CA 15-3 and lung function indicators of disease severity: FVC%, DLCO%, and SpO$_2$-Ex.

A prospective study evaluated 85 patients and found higher serum levels of CA 15-3 in patients with FVC% < 50%.[26] In the present study, there was a strong correlation of serum levels of CA 15-3 with gas exchange variables, DLCO%, and SpO$_2$-Ex, suggesting that CA 15-3 was an indicator of the extent of alveolar damage.

One study measured CA 15-3 levels in 84 patients with systemic sclerosis and ILD and found that such levels strongly correlated with semiquantitative HRCT scores.[9] Jacob et al.[19] studied patients with HP and found that CALIPER quantitative analysis was more accurate than visual HRCT scores and that there was a better correlation with functional and morphological variables. In the present study, the quantitative analysis of HRCT findings was carried out using LTA and CALIPER. There was a direct correlation between serum levels of CA 15-3 and the following HRCT findings: total ground-glass opacities, total honeycombing, and total fibrosis score (i.e., total reticular opacities plus honeycombing). One study evaluated patients with
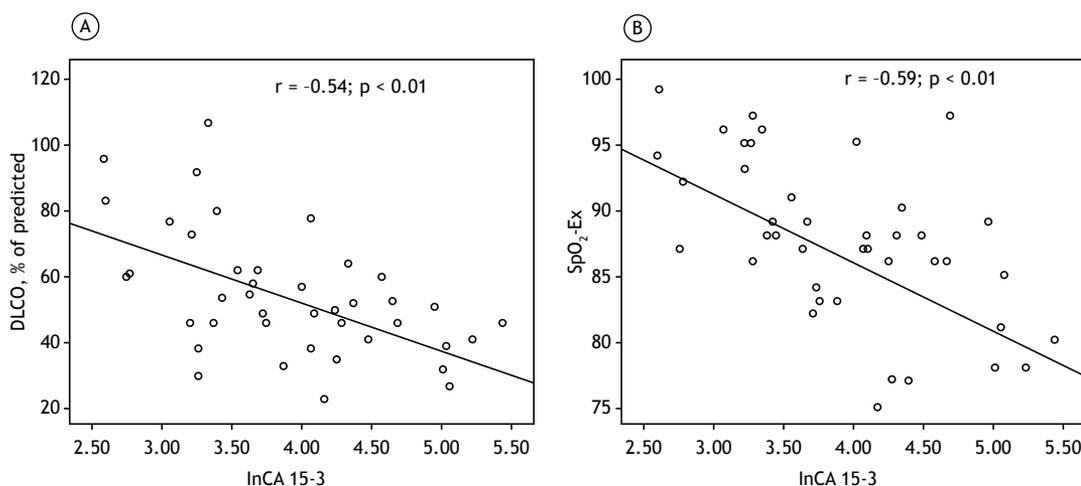
Figure 2. Correlation of natural logarithm (ln) of serum levels of carbohydrate antigen (CA) 15-3 with DLCO in % of predicted (in A) and SpO$_2$ at the end of a 4-min step test (SpO$_2$-Ex).

Table 2. Correlation between carbohydrate antigen (CA) 15-3 levels and tomographic findings.[a]

HRCT finding	Spearman's correlation coefficient	p
Total ground-glass opacities	0.54	0.001
Total hyperlucent areas	0.35	0.03
Total honeycombing	0.36	0.03
Total fibrosis score	0.34	0.04
Total findings	0.47	0.004

[a]CALIPER quantitative analysis.
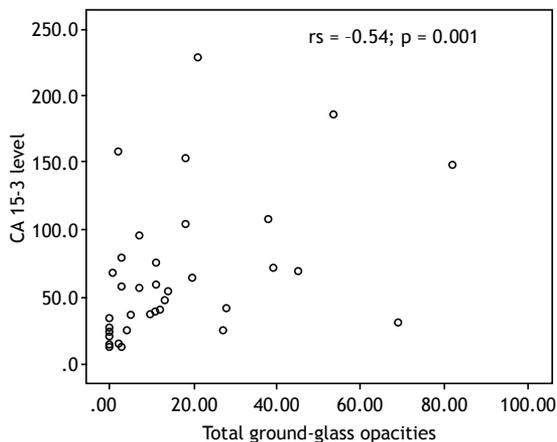
Figure 3. Correlation between serum levels of carbohydrate antigen (CA) 15-3 and computerized quantitative analysis of total ground-glass opacities.

IPF who underwent lung transplantation and showed that CA 15-3 levels decreased after the procedure.[28] This result corroborates the relationship between CA 15-3 levels and the extension of ILDs.

There are limitations in the present study. In 8 cases, HRCT was not performed in our hospital, and this resulted in the use of different tomographic techniques. However, those images were considered adequate for CALIPER analysis. Former smokers were not excluded, and that might have affected some functional and tomographic findings. However, serum levels of CA 15-3 were similar between nonsmokers and former smokers. In addition, KL-6 quantitative assays were unavailable, and, therefore, no comparisons between KL-6 and CA 15-3 levels were made.

When a candidate biomarker is identified, it should be easily measurable and mechanistically plausible. It should be validated in another study and undergo biological testing to establish its role in the pathogenesis of a disease.[22]

In conclusion, CA 15-3 is likely to be a biomarker of disease severity in patients with cHP, particularly regarding gas exchange abnormalities.

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AUTHOR CONTRIBUTIONS

RGF: histological analysis of the study and approval of the final version. MRS, ABB, and RBM: study design, manuscript drafting, and approval of the final version. MFMLM and GSPM: analysis of HRCT images and approval of the final version. CACP: coordination, statistical analysis, manuscript drafting, and approval of the final version. PSG: study design, data collection, manuscript drafting, and approval of the final version.

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