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Original article

Carbohydrate antigen 15.3 as a serum biomarker of interstitial lung disease in systemic sclerosis patients

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ABSTRACT

Background: To determine the usefulness of Ca 15.3 as a candidate biomarker in systemic sclerosis (SSc) patients with interstitial lung disease (ILD).
Methods: Two-hundred-twenty-one SSc patients with Ca 15.3 determinations were considered; 168 had evidence of interstitial lung involvement on high-resolution computed tomography (HRCT); digitalized scans were available for scoring in 84 subjects. Discrimination between patients with or without ILD, was assessed by receiving operating characteristics (ROC) analysis; correlations between HRCT scores and Ca 15.3 were performed. Survival and serial pulmonary function tasting (PFT) data were used for prognostication.
Results: Ca 15.3 serum levels strongly correlated with HRCT scores ($r = 0.734$, $p < 0.0001$) which were predictors of survival at the 20% threshold ($p = 3.1 * 10^{-4}$). Ca 15.3 had an area under ROC to detect the meaningful 20% fibrosis extent equal to 0.927 and abnormal Ca 15.3 values were capable of differentiating between patients at hi- or low-risk for progression in the group with undetermined disease extent (HR = 3.209, confidence interval [CI_{95}] = 1.56–6.602, $p = 0.002$). Ca 15.3 outperformed other PFT measures in providing a separation of survival estimates where HRCT scans are unavailable. The combined use of HRCT scores and Ca 15.3 in SSc–ILD patients was more discriminatory (HR = 4.824, CI_{95} = 2.612–8.912, $p < 0.0001$) than the staging system based on HRCT scores plus FVC (HR = 2.657, CI_{95} = 1.703–4.147, $p < 0.0001$) and characterized by lower prediction errors (0.2134 vs 0.2234).
Conclusion: Ca 15.3 is a rapid and inexpensive candidate biomarker for SSc–ILD being proportional to the extent of lung injury and specific and sensitive in assessing meaningful extents of the disease with prognostic significance.

1. Introduction

Systemic sclerosis (SSc) is a multi-system autoimmune disorder; characterized by autoantibody production, endothelial damage with obliterative microvascular disease, inflammation and fibrosis affecting the skin and internal organs [1,2] Pulmonary involvement is a common manifestation in SSc which presents either as interstitial lung disease (ILD) or pulmonary arterial hypertension (PAH) [3] and it constitutes nowadays the leading cause of disease-related morbidity and mortality in scleroderma patients [4].

The quantification of ILD extent is usually performed by high-resolution computed tomography (HRCT) whose overall scores correlate with survival [5]. Similarly, pulmonary function testing (PFTs) have a prognostic relevance in SSc patients, with poor prognosis associated with a reduction in the forced vital capacity (FVC) predicted values [5]. ILD is believed to be the consequence of alveolitis and the results from a placebo-controlled trial indeed indicate that untreated active alveolitis leads to a progressive deterioration of lung function [6]. The early identification and staging of ILD is therefore of paramount importance to the management of SSc patients. From a clinical point of view there's still an unmet need for non-invasive biological markers (biomarkers) that can be routinely used to herald the presence of SSc–ILD, its extent, progression and, possibly, its activity.

To date, several biomarkers have been tested in pulmonary fibrosis [7], including patients with SSc: the surfactant proteins A and D (SP-A, SP-D) [8–12], the Krebs von den Lungen 6 Antigen (KL-6) [8–12], the CCL18 chemokine [11] and the serum clara cell 16-kDa (CC16) protein [12]. KL-6, a high molecular weight glycoprotein classified in the category of cluster 9 mucin-1 (MUC1) of lung tumor and differentiation antigens [13,14] is perhaps the most widely studied biomarker in lung fibrosis either correlated with SSc or not [7]. Some reports suggest that other mucins [15], may however have a role in the pathogenesis of pulmonary fibrosis and that may possibly be a used as a marker for ILD occurrence [16,17] or disease activity [18]. Among those, the most promising is the carbohydrate antigen 15.3 (Ca 15.3), a product of the same MUC1 gene that encodes for KL-6; both KL-6 and Ca 15.3 exist in different positions of the

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high-weight glycoprotein MUC1 which expressed on the surface of various epithelial cells, including type II pneumocytes [15]. Of interest, in a preliminary report on 42 SSc patients, high levels of Ca 15.3 have been described in subjects with no evidence of malignancy and have been associated to the presence of severe lung involvement [19].

The present study was designed to determine the role of Ca 15.3 as a biomarker of SSc–ILD. Firstly, we evaluated the ability of Ca 15.3 to discriminate between patients with or without a HRCT-proven diagnosis of ILD. Secondly, we evaluated whether Ca 15.3 levels could be directly proportional to the disease extent on HRCT. Thirdly we assessed the prognostic significance of the Ca 15.3 antigen. Finally we showed that Ca 15.3 could integrate HRCT scores to categorize patients at risk for ILD progression and poor prognosis.

2. Methods

2.1. Patients

Data from 221 consecutive SSc patients attending our outpatient clinic with serum determination of Ca 15.3 were considered and retrospectively collected. To be included into the study all the patients had to: 1) have a diagnosis of SSc according to the preliminary criteria for the classification of SSc proposed by the American College of Rheumatology (ACR) [20] with no sign or symptoms of overlap features; 2) have no evidence of breast cancer detected by mammography or by breast ultrasound; 3) have no evidence of hepatic cirrhosis or pancreatic diseases detected by abdominal ultrasound [21]; 4) have no personal history of breast cancer, viral or autoimmune hepatitis, hepatic cirrhosis or pancreatic diseases; and 5) have no indirect signs of pulmonary arterial hypertension on echocardiography [22].

All the eligible patients were divided into three categories: 1) patients with concurrent determination of Ca 15.3 and digitalized HRCT scans available for scoring and with evidence of lung fibrosis (n = 84) (training ILD group); 2) patients with concurrent determination of Ca 15.3 and digitalized HRCT scans available for scoring and without evidence of lung fibrosis (n = 53) which served as negative controls; and 3) patients followed at our center for SSc–ILD but without digitalized HRCT scans available for scoring (n = 84) which served as validation cohort for survival analysis (testing ILD group).

All the patients were categorized as having the limited cutaneous (lcSSc) or the diffuse cutaneous (dcSSc) subset of the disease, according to LeRoy et al. [23]. Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on Hep_2 cells (Kallestad, Chaska, MN) [24]; extractable nuclear antigens (ENAs) were determined by a commercial enzyme-linked immunoassay (ELISA) (Diamedix, Miami, FL).

Serum Ca 15.3 levels were determined at the Central Laboratory Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, via an Immunoradiometric assay (IRMA) kit (Diasource, Belgium) according to the producer's protocol.

All the patients at the time of referral gave their written consent to have their clinical and laboratory data collected and used for epidemiological studies; the study protocol was approved by the local ethic committee.

2.2. HRCT scanning techniques and scoring

HRCT scans were performed with a collimation of 1.5–3.0 mm and an interspace of 10 mm; images were reconstructed with a high-spatial-frequency algorithm and digitalized at window settings appropriate for viewing lung parenchyma (window center, −550 HU; window width, 1500 HU).

HRCTs were scored according to Goh et al. [5] by two observers (LB, AlS) who were blinded to the clinical data. Scans were reviewed at the following five levels: 1) origin of great vessels; 2) main carina; 3) pulmonary venous confluence; 4) between levels 3 and 5; and 5) immediately above the right hemidiaphragm. The overall extent of interstitial lung disease was estimated at each level. Discrepancies in the extent of ILD more than 20% at any level were reviewed jointly and resolved with consensus evaluation. For each patient, the extent of ILD was derived by averaging the scores at each level, as assessed by the two observers; the mean value was used in analysis.

2.3. Survival and disease progression

Both the prognostic significance of HRCT scores and CA 15.3 serum levels were tested using the composite endpoint previously used by Goh et al. [5]. Mortality, disease progression, defined as a decline in either FVC levels of ≥10% from baseline or DLco levels of ≥15% from baseline, and "progression-free survival" were quantified from the date of Ca 15.3 determination up to 5 years; only ILD-related deaths were considered for survival analysis. In our center, PFTs are per clinical practice sequentially evaluated at a 2–6 months intervals, the monthly use of immunosuppressants (cyclophosphamide [CYC], micophenolate, azathioprine) and the average daily dose of steroids were recorded.

2.4. Statistical analysis

Statistical differences among the three study groups were assessed by one-way analysis of variance (ANOVA) with Dunnet's T3 post-hoc algorithm for continuous covariates or by means of chi-square test with Bonferroni adjustment for categorical variables.

To determine the best Ca 15.3 threshold capable of differentiating between patients with or without ILD, receiving operating characteristics (ROC) analysis was performed and the area under ROC curves (AUROC) was calculated; the optimal cutpoint was chosen among the coordinates of the ROC curves as the one that maximized the Youden index (J statistic) that is the point that detects the farthest point from chance and gives a balanced weight between sensitivity and specificity [25,26]. The procedure was performed considering any degree of fibrosis or at least the 5%, 10%, 15%, and 20%, of lung involvement as the target variable.

Correlations among Ca 15.3 levels, the extent of ILD disease on HRCT and the other lung parameters were performed by means of Pearson's correlation coefficient except in the presence of skewed variables where Spearman's rho was used; 95% confidence intervals were estimated by 10,000 bootstrap replicates.

Univariate Cox regression analysis with time-dependent covariates was used to determine which factors were associated with progression-free survival, after checking that the principle of the proportionality of hazards was not violated.

To determine the optimal cutpoint in HRCT scores or in CA 15.3 serum levels to discriminate SSc–ILD patients with different survival estimates, the method proposed by Contal and O'Quigley [27] was applied. The optimal Ca 15.3 cutpoint found in the training ILD group was then used to categorize patients in the testing ILD group (group 3) and to assess their survival; this categorization was compared against the 70% FVC threshold proposed by Goh et al. [5] to categorize patients with undetermined extent of lung involvement. Lastly, we validated the staging system described by Goh et al. [5], based on the 20% HRCT threshold and 70% FVC threshold, in all the patients with SSc–ILD (group nos. 1 and 3); an alternative model which uses HRCT scores and Ca 15.3 to categorize patients was also assessed in the same population. Hazard ratios were used to compare the categorization into hi- and low-risk groups. Besides that, we assessed the prognostic significance of the two alternative models by means of the prediction error: the Brier score for censored data in its integrated version (IBS), where lower values indicate a better performance of the model [28]. To better gauge the extent to which covariates determine the progression-free survival, we also expressed the results as explained residual variation (V), that is the relative gains in predictive accuracy when prediction based on covariates replaces unconditional predictions [29].