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Matrix metalloproteinases and airway remodeling and function in primary ciliary dyskinesia

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ABSTRACT

Background: The balance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) is important in the regulation of airway damage.
Objective: To evaluate whether they are important in the pathophysiology of primary and secondary ciliary dyskinesia (PCD, SCD).
Methods: We measured sputum bacteriology, lung CT changes, MMPs, TIMPs and lung function in 86 patients (51 PCD, 35 SCD) in a cross-sectional study; the 10 controls studied did not have HRCT or sputum cultures. MMPs, TIMPs and lung function were evaluated longitudinally for up to one year in 38 PCD patients.
Results: At baseline, there were no differences in MMPs, TIMPs and MMPs/TIMPs, between PCD and SCD but lower levels were found in controls. There was an association between poorer lung function with increasing levels of MMPs in PCD, while in SCD only MMP-9/TIMP-1 values correlated with FRC z-scores. Levels of MMPs and TIMPs significantly correlated with severity HRCT changes. Longitudinally, there were significant correlations between slope of changes in spirometric parameters and slope of change in sputum MMPs in PCD patients.
Conclusions: In conclusion, we report for the first time that increased MMPs are associated with worse airway damage in PCD and SCD, and thus are potential therapeutic targets.

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1. Introduction

Primary ciliary dyskinesia (PCD) is a disorder clinically characterized by recurrent-persistent respiratory tract infection and inflammation [1], with higher levels of sputum neutrophil chemoattractants than in cystic fibrosis (CF) [2,3].

Worsening of lung function due to progressive damage of airways and lung parenchyma, and consequent widespread bronchiectasis, bronchiolectasias, fibrosis, and emphysema are classical long-term manifestation of the disease.

These structural airway wall changes may occur secondary to infection and neutrophilic inflammation with an abnormal extracellular matrix (ECM) degradation and deposition [4].

It is known that in CF [5], and also in post-infectious bronchiectasis [6,7], that there are changes of ECM proteins with an imbalance between metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). In particular, matrix metalloproteinase-8 (MMP-8), matrix metalloproteinase-9 (MMP-9), and gelatinase B have

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been found to be elevated in the airway secretions of patients with CF [5]. Only one study has been performed in a limited number of PCD patients [8]. Thus our aim was to determine, in a larger group of patients, the relationships between these MMPs and their corresponding TIMPs with structural alterations of the airways-lung parenchyma, with lung function, and with bacterial infection.

Furthermore, we evaluated longitudinally whether imbalances between MMPs and TIMPs are related to further impairment of lung function or bacterial infections in PCD patients, in order better to try to understand the pathophysiology of PCD, and open potential novel opportunities for treatment.

2. Materials and methods

2.1. Design

At enrolment into the study sputum MMPs and the corresponding TIMPs, lung function measurements, chest high-resolution computed tomography (HRCT), and sputum culture were performed in all patients consecutively evaluated for recurrent lower airway infections and diagnosed as affected by PCD or secondary ciliary dyskinesia (SCD). In addition, PCD patients were prospectively followed up to one year with periodic evaluation of sputum MMPs, TIMPs, lung function measurements and sputum culture.

Informed consent was obtained from adult patients or from the parents of children, and age-appropriate assent from the children.

The study protocol was approved by the local Hospital Ethical Committee.

2.2. Subjects

For one year all subjects aged ≥6 years, with a diagnosis of PCD made on standard criteria [9,10] or SCD (in these patients, PCD was excluded as previously reported [11–13]) were consecutively enrolled in the Department of Paediatrics of the University of Pisa. In all patients, the diagnosis was performed by ciliary motion analysis, transmission electron microscopy evaluation, and ciliogenesis in culture study [9–13]. Diagnostic details are on-line. A small group of control healthy subjects was also evaluated in the same period.

2.3. Lung function

All lung function testing (Master Screen Body equipment; Jaeger, Wuerzburg, Germany) was performed utilising standard American Thoracic Society methodology [14,15]. See details on-line.

2.4. CT scanning of chest

Chest HRCT was performed using the same scanner (Multislice CT; General Electric Medical Systems, Milwaukee, Michigan, USA). Slices (1 mm thick) were obtained with 10 mm spacing in the supine position. All images were evaluated by the same radiologist (DC), unaware of the clinical data, and scored by a modified Bhalla system [16]. Details can be found on-line.

2.5. Sputum collection

Sputum samples were collected in each patient by spontaneous expectoration or by induction through the use of positive expiratory pressure (PEP) mask without using hypertonic saline inhalation. One part of the sample was sent for microbiological examination, while the other was diluted 1:2 with 0.9% normal saline and centrifuged at 3000 rpm for 10 min. The resulting cell-free supernatants were aliquoted and stored frozen at −70 °C for subsequent biological markers analysis.

Dithiothreitol was not used at any stage of processing.

2.6. MMPs activity and TIMPs quantification assays

MMP-8 and MMP-9 and TIMP-1, the natural inhibitor of MMP-9, and TIMP-2, were measured by ELISA (R&D Systems, Minneapolis, MN) [17] by a fully automated DSX system (Technogenetics, Sesto S. Giovanni, Italy). The results were expressed in ng/mL.

2.7. Statistical analysis

There are no available data to do a power calculation, so the sample size was opportunistic. Baseline variables were expressed as group mean ± SD or as median and IQR when the variables were non-normally distributed. To allow comparison of observations from different normal distributions, all lung function data were expressed as SD (z) scores of the reference population [18].

In each patient followed prospectively for one year, slopes of change in sputum MMPs, TIMPs, their ratios and slopes of decline in lung function were calculated using linear regression analysis. The relationship between categorical variables (i.e. increase or decrease in slopes of change in sputum MMPs, TIMPs, their ratios and/or in slopes of decline in lung function and/or presence or absence of infection) were measured using chi-square test.

Differences between means were evaluated by the Student *t*-test. Differences between groups were evaluated by the Kruskal – Wallis (non-parametric) test and, when significant differences were found, Mann-Whitney *U* test for between-groups comparisons with Bonferroni correction for multiple comparisons was employed [19]. Correlations between continuous non-normally distributed variables were assessed using Spearman's rank correlation coefficients.

A p value < 0.05 was considered statistically significant. All statistical calculations were performed using SPSS V. 23.0 for Windows.

3. Results

3.1. Cross-sectional evaluation

Demographic, clinical and laboratory characteristics of the patients are shown in Table 1. Eighty-six patients (51 PCD, 35 SCD) and ten controls were studied. Twenty-four (47%) out 51 subjects with PCD were children (age range 6–17 years, median 12.4 years; IQR 5.1) and 27 adults (age range 18–51 years, median 35.1 years; IQR 11.6). Of the 35 subjects with SCD 11 (31%) were children (age range 6–17 years, median 13.2 years; IQR 4.8) and 24 were adults (age range 18–62 years, median 33.7 years; IQR 12.2).

There were no differences in MMPs, TIMPs and MMPs/TIMPs between PCD and SCD while highly significantly lower levels were found in controls.

There were no differences in lung function results between PCD and SCD patients with only z-score for TLC significantly higher in patients with primary compared with secondary disease ($p = 0.003$).

Bronchiectasis was documented in 44 PCD patients, respectively in 25 (93%) adults and 19 (79%) children, and severity class at HRCT Bhalla score was 0 in 6 PCD patients, 1 in 10, 2 in 26, and 3 in the remaining 9 subjects. Bronchiectasis was documented also in 29 SCD patients, respectively in 19 (79%) adults and in 10 (91%) children with a severity class at HRCT Bhalla score of 0 in 6 SCD patients, 1 in 10, 2 in 10, and 3 in the remaining 9 subjects with no significant different prevalence between PCD and SCD patients.

At enrolment, infection with *Pseudomonas aeruginosa* (alone or