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The heterogeneity of systemic inflammation in bronchiectasis

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ABSTRACT

Background: Systemic inflammation in bronchiectasis is poorly studied in relation to aetiology and severity. We hypothesized that molecular patterns of inflammation may define particular aetiology and severity groups in bronchiectasis.

Method: We assayed blood concentrations of 31 proteins from 90 bronchiectasis patients (derivation cohort) and conducted PCA to examine relationships between these markers, disease aetiology and severity. Key results were validated in two separate cohorts of 97 and 79 patients from other centres.

Results: There was significant heterogeneity in protein concentrations across the derivation population. Increasing severity of bronchiectasis (BSI) was associated with increasing fibrinogen (rho = 0.34, p = 0.001 −validated in a second cohort), and higher fibrinogen was associated with worse lung function, *Pseudomonas* colonisation and impaired health-status. There were generally similar patterns of inflammation in patients with idiopathic and post-infectious disease. However, patients with primary immunodeficiency had exaggerated IL-17 responses, validated in a second cohort (n = 79, immunodeficient 12.82 pg/ml versus idiopathic/post-infectious 4.95 pg/ml, p = 0.001), and thus IL-17 discriminated primary immunodeficiency from other aetiologies (AUC 0.769 (95%CI 0.661−0.877)).

Conclusion: Bronchiectasis is associated with heterogeneity of systemic inflammatory proteins not adequately explained by differences in disease aetiology or severity. More severe disease is associated with enhanced acute-phase responses. Plasma fibrinogen was associated with bronchiectasis severity in two cohorts, *Pseudomonas* colonisation and health status, and offers potential as a useful biomarker.

1. Introduction

Bronchiectasis is a neglected chronic inflammatory airway condition that is the end anatomical result of diverse aetiological insults. Whilst it has been known for some time that bronchiectasis is also associated with systemic inflammation [1], the nature of this response has not been well studied in relation to bronchiectasis aetiology and severity. Importantly, no previous studies have examined whether inflammation differs between major causes of bronchiectasis such as idiopathic and post-infectious disease. Indeed, there are no existing data examining a comprehensive panel of inflammatory proteins in the systemic compartment of patients with bronchiectasis.

Current concepts of bronchiectasis severity emphasise the importance of multi-component assessment [2,3]. Scores such as the Bronchiectasis Severity Index (BSI) [2] and FACED [3] have been applied to either predict mortality and/or morbidity such as, hospital admissions, exacerbation frequency and quality of life. There is also a paucity of data examining causes of death in bronchiectasis despite a suggestion of increased cardiovascular risk [4]. This is a concept well established in other chronic airway diseases such as chronic obstructive pulmonary disease (COPD) and which may relate to systemic inflammation [5]. There is therefore the need to examine how bronchiectasis severity is associated with systemic inflammation and, in particular, which components of bronchiectasis severity drive this association.

We designed a study to address the hypotheses that systemic inflammatory markers can distinguish aetiology and severity groups in bronchiectasis, employing an unsupervised principal component analysis. In doing so we have conducted the largest and most comprehensive analysis of systemic inflammatory proteins in

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bronchiectasis ever reported. Moreover, we validated key results in two replication cohorts. Our results inform on the heterogeneity of systemic inflammatory responses in bronchiectasis with regard to aetiology and severity of disease.

2. Method

90 patients with stable, clinically significant bronchiectasis – defined as regular sputum production and/or recurrent respiratory infections with a diagnostic computed tomography (CT) scan - were recruited as a convenience sample from out-patient clinics at the Royal Free London and University College London Hospitals NHS Foundation Trusts, UK. Patients with a primary clinical diagnosis of COPD were excluded. These patients formed the derivation cohort. Patients were only recruited if free from exacerbation treated with antibiotics for one month or more. Patients provided written informed consent, and the study was approved by Hampstead Research Ethics Committee (10/H0720/43).

2.1. Clinical assessment

For the purposes of this analysis, information was collated to permit allocation of patients into aetiological groups according to the British Thoracic Society guideline [6]. Idiopathic disease was defined as the absence of an alternative more plausible cause of bronchiectasis.

Bronchiectasis severity was first assessed using the Bronchiectasis Severity Index [2] and therefore data on age, body mass index (BMI), most recent lung function (forced expiratory volume in 1 s, FEV_1), self-reported hospitalisation (previous two years) and exacerbations (previous year), Medical Research Council (MRC) dyspnoea scale, the presence of colonising organisms on routine sputum culture and radiological extent of disease on the most recent CT scan were collated and entered into the online BSI calculation tool: http://www.bronchiectasisseverity.com/. Mild bronchiectasis was defined as a score of 0–4, moderate as 5–8 and severe as ≥9 units. To provide further information in relation to bronchiectasis severity, we also calculated the alternative FACED bronchiectasis mortality score [3], which includes age, FEV_1, lobar involvement, dyspnoea (mMRC score) and *Pseudomonas* colonisation status. The FACED score is as follows: mild 0–2 points, moderate 3–4 points and severe 5–7 points.

2.2. Assessment of serum proteins

Venous blood samples were drawn. A citrated plasma sample was analysed for fibrinogen using the Clauss method (IL ACL Top Coagulation Analyzer; Instrumentation Laboratories, Lexington, MA). A serum sample was analysed for C-Reactive Protein using a Modular Analytics E 170 Module (Roche, Burgess Hill, UK). A second serum sample was centrifuged (10 min, 2000 rpm, $4^{O}C$) with the supernatant then stored at $-80^{O}C$ for later analysis of the remaining 29 mediators (see Table 1). These were assessed using the Meso Scale Discovery Platform 'V-PLEX Human Cytokine 30-Plex Kit' (Meso Scale Discovery, Rockville, MD, US) according to the manufacturer's instructions.

2.3. Validation cohorts

Key results were validated in two samples of patients from specialist bronchiectasis clinics in Dundee and Newcastle, UK. These patients had clinically significant bronchiectasis and also underwent standardised testing according to BTS recommendations, as in the derivation cohort. Ethical approval was granted (12/ES/0059 and 12/NE/0248 respectively). Fibrinogen was measured by the Clauss method (Dundee patients). Serum IL-17 (Newcastle patients) was measured using a commercial quantitative enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer's protocol (DuoSet® ELISA Development System, human IL-17, R&D Systems, UK). Health status as assessed by the St. George's Respiratory Questionnaire (SGRQ) was available in the Dundee patients.

Table 1
Results of the analysis of 31 proteins in 90 patients with bronchiectasis from the derivation cohort. All results are expressed as geometric mean (SD) pg/ml except Fibrinogen (g/l) and CRP (mg/l).

(all logs)	n = above the lower limit of detection	Mean (SD)
CRP	90	2.8 (3.9)
Eotaxin	90	147.9 (1.9)
Eotaxin-3	73	14.1 (3.3)
Fibrinogen	90	3.5 (1.4)
GM-CSF	83	0.4 (2.3)
IFN-γ	84	9.1 (3.4)
IL-1α	41	0.4 (6.2)
IL-1β	53	0.1 (4.3)
IL-2	45	0.3 (4.3)
IL-4	37	0.1 (3.4)
IL-5	86	0.5 (2.6)
IL-6	86	1.3 (2.5)
IL-7	90	15.1 (1.6)
IL-8	90	13.5 (2.8)
IL-10	78	0.4 (3.2)
IL-12p40	90	107.2 (2.2)
IL-12p70	65	0.7 (2.3)
IL-13	63	6.9 (1.7)
IL-15	90	2.3 (1.4)
IL-16	90	257.0 (1.6)
IL-17	90	4.0 (2.6)
IP-10	90	309.0 (2.5)
MCP-1	90	263.0 (1.8)
MCP-4	90	81.3 (1.6)
MDC	90	831.8 (1.7)
MIP-1α	83	21.9 (1.6)
MIP-1β	90	112.2 (1.6)
TARC	90	275.4 (2.1)
TNF-α	90	1.8 (1.6)
TNF-β	79	0.2 (2.8)
VEGF	90	109.6 (1.9)

Values below the lower limit of detection were assigned a value of half that lower limit.

2.4. Statistical analysis

Statistical analysis was performed in IBM SPSS version 22 (IBM Corporation, Armonk, NY, US). Data were tested for normality using the Kolmogorov-Smirnov test. Clinical data are reported as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate.

The majority of the serum proteins were not normally distributed and therefore all were $\log_{10}$ transformed for further analysis. As described in Table 1, any assays below the detection limit were assigned a value of half that lower limit of detection (LLD). 17 missing fibrinogen results in the derivation cohort arising because no plasma sample was available were single-imputed based on a significant correlation between fibrinogen and CRP (rho = 0.572, $p < 0.001$). We validated a key finding in relation to fibrinogen using minimum, mean and maximum values derived from multiple imputation using the 'mi' command in STATA version 14 (www.stata.com).

We used Principal Component Analysis (PCA) [7] to assess variation in the proteins across the population. The protein most closely correlated with each of the first five principal components