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Relationship between disease severity and inflammatory markers in cystic fibrosis

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Abstract

To evaluate the clinical use of measuring neutrophil, lymphocyte, and eosinophil activities, serum myeloperoxidase (MPO), soluble interleukin-2 receptors (sIL-2R), and eosinophil cationic protein (ECP) were measured in 98 patients with cystic fibrosis and in 85 healthy children. Serum concentrations of MPO, sIL-2R, and ECP were increased in patients with cystic fibrosis (median 807 µg/l, 4452 pg/ml, 48.8 µg/l, respectively) compared with the controls (median 319 µg/l, 2743 pg/ml, 9.4 µg/l). ECP concentrations, but not serum MPO or sIL-2R, were significantly related to disease severity assessed by the Shwachman-Kulczycki score and by pulmonary function (forced expiratory volume in one second % predicted). Neither ECP nor sIL-2R was influenced by *Pseudomonas aeruginosa* infection, acute pulmonary exacerbation, or atopy. Serum MPO, however, was strongly correlated with acute pulmonary exacerbation. In the light of these findings the measurement of serum ECP might thus be used for clinical monitoring and for assessing disease severity in cystic fibrosis. The measurement of serum MPO and sIL-2R did not correlate with the disease severity.

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The most frequent clinical manifestation in patients with cystic fibrosis is progressive pulmonary destruction due to chronic endobronchial infection. There is increasing evidence that immune mediated inflammation also contributes to progressive pulmonary tissue damage. Knowledge about the immune processes in cystic fibrosis allows us to analyse and quantitate cells or cell products suggested to be involved in the pathology and to follow changes as a reflection of pulmonary disease in cystic fibrosis.[1-4] Eosinophils appeared to play a minor part until the demonstration of highly activated eosinophils measured by eosinophil cationic protein (ECP) concentrations in patients with cystic fibrosis.[1 2] In addition, assessment of pulmonary inflammation is possible by measuring cell products in blood samples.[1 2 5] Persistence of endobronchial infection may cause an excessive immune response,[6] reflected by high concentrations of immunoglobulins, immune complexes, and neutrophil products—for example, elastase and myeloperoxidase (MPO).[7] Concentrations of soluble interleukin-2 receptors (sIL-2R), a marker of T lymphocyte activation, have been shown to be increased even before any clinical evidence of lung inflammation due to infection in cystic fibrosis.[3 4] Thus pulmonary manifestations in cystic fibrosis may be considered as an inflammatory disease.

In this study, the activation of neutrophils, lymphocytes, and eosinophils was examined by measuring concentrations of MPO, sIL-2R, and ECP in serum samples from healthy subjects and from patients with cystic fibrosis of variable disease severity to determine their role in the assessment of the clinical disorder.

Patients and methods

PATIENTS AND CONTROLS

Ninety eight patients with cystic fibrosis from the Cystic Fibrosis Care Center Vienna were studied (mean (SD) age 11.0 (7.69) years; 45 boys and 53 girls). Fifty four patients were infected with *Pseudomonas aeruginosa*, 68 with *Staphylococcus aureus*, and 46 with *Haemophilus influenzae*, as determined by sputum cultures. The diagnosis of acute pulmonary exacerbation in 39 patients with cystic fibrosis was defined as a marked increase of C reactive protein (median 86 mg/l), by weight loss, anorexia, increased cough, increased sputum production, fever with and without new lung infiltrates, and deterioration of oxygen saturation and pulmonary function. Atopy was present in 31 patients and of these 22 were sensitised against *Aspergillus fumigatus*. A patient was considered atopic if total serum IgE antibody levels (median total serum IgE 456 *v* 22 kU/l; $p < 0.0001$) were increased (above the age dependent normal values) and if specific IgE antibodies ($\geq$ class 2) against more than one allergen could be detected. None of the patients had received steroids within a month before drawing blood.

Eighty five healthy non-atopic subjects (10.8 (5.68) years) with normal total IgE concentrations (median serum IgE 24 kU/l) were recruited as controls. Blood was obtained at routine sampling for clinical evaluation.

ASSESSMENT OF DISEASE SEVERITY AND PULMONARY FUNCTION

Disease severity was assessed by the Shwachman-Kulczycki score,[8] which in our setting was limited to a maximum of 75 (excluding radiography). The following pulmonary function tests were performed in 67 patients with cystic fibrosis: forced vital capac-

Table 1 Influence of acute pulmonary exacerbation and P aeruginosa infection on serum levels of ECP, MPO, and sIL-2R in 98 patients with cystic fibrosis

	Acute pulmonary exacerbation			*P aeruginosa infection*		
	With (n=39)	*Without (n=59)*	*p Value*	*With (n=54)*	*Without (n=44)*	*p Value*
ECP (μg/l)	61.6 (23.4-75.8)	44.9 (14.8-65.0)	NS	48.0 (28.5-76.6)	49.8 (13.8-66.9)	NS
MPO (μg/l)	1342 (1003-1769)	626 (495-804)	< 0.0001	1007 (588-1780)	698 (495-798)	< 0.001
sIL-2R (pg/ml)	5040 (4116-7014)	4410 (3570-6804)	NS	4452 (3780-6840)	4536 (3528-6762)	NS

Results are presented as median (quartile 1 – quartile 3); p values indicate significant differences between the groups (Mann-Whitney U test). NS = not significant.

Table 2 Correlation (r) of ECP, MPO, and sIL-2R and various clinical parameters

	ECP	*MPO*	*sIL-2R*
Shwachman score	−0.613; p < 0.0001	−0.197; p < 0.005	0.080; NS
Forced vital capacity	−0.493; p < 0.0001	−0.227; p < 0.01	0.048; NS
FEV_1 (%)	−0.532; p < 0.0001	−0.195; p < 0.05	0.102; NS
MEF_{50} (%)	−0.475; p < 0.0001	−0.227; p < 0.01	0.083; NS
IgE	0.121; NS	0.099; NS	0.009; NS

Correlation was calculated by means of the Kendall Tau B test. NS = not significant.

ity, forced expiratory volume in one second (FEV_1), and maximum expiratory flow at 50% of vital capacity (MEF_{50}). These were recorded in the form of a maximum flow volume curve (Masterlab, Jaeger, Germany) according to the American Thoracic Society standard.[9] Results were presented as a percentage predicted based on accepted reference standards.[10]

BLOOD SAMPLES

The cell numbers were counted using an automated haematology analyser (Sysmex NE-5500, Müller GesmbH, Austria) with coefficients of variation for eosinophils less than 7%.[1] The ECP/MPO ratios were determined using specific and sensitive radioimmunoassays (Pharmacia, Sweden) in duplicate.[11 12] In brief, ECP/MPO ratios in serum samples compete with a fixed amount of ECP/MPO labelled with ^{125}I for the binding sites of specific antibodies. sIL-2R levels were assessed by immunoenzymometric assays (Immunotech, France) in which monoclonal antibodies directed against two different epitopes of sIL-2R were used.[4] All methods showed interassay coefficients of variation lower than 10%.

STATISTICAL ANALYSIS

Results are expressed as median (quartile 1 - quartile 3) unless stated otherwise. Non-parametrical statistical tests were used in the comparative analysis. Each pairing was examined using the Mann-Whitney U test. Correlation coefficients (r) were obtained by using the Kendall Tau B method. The level of significance was considered at $p < 0.05$.

Results

CHARACTERISTICS OF PATIENTS

Three patients with cystic fibrosis had severe disease according to the Shwachman scoring system, 17 had moderate, and 41 had mild disease. Twenty nine patients had a good and eight a very good clinical status. The forced vital capacity was 60.4% (46.2–88.2%) predicted, the FEV_1 was 69.8% (54.8–81.1%) predicted, and the MEF_{50} was 41.0% (13.0–70.4%).

EOSINOPHIL COUNTS AND SERUM ECP

Eosinophil counts did not differ between patients with cystic fibrosis (220 (160–301) cells/μl) and the control subjects (245 (143–368) cells/μl), whereas serum ECP concentrations were increased (48.8 (16.5–68.2) *v* 9.4 (6.6–12.0) μg/l; $p < 0.0001$). Eosinophil counts and ECP levels from atopic patients with cystic fibrosis (median 229 cells/μl and 54.6 μg/l, respectively) were also not different from those of the non-atopic patients (median 220 cells/μl and 44.9 μg/l, respectively). In addition, eosinophil counts and serum ECP concentrationss (table 1) were not influenced by *P aeruginosa* infection (median eosinophil counts of infected subjects 208 cells/μl *v* non-infected patients 226 cell/μl) and acute pulmonary exacerbation (median eosinophil counts 198 *v* 220 cells/μl). Division of the data into classes among the Shwachman score indicated a relation of serum ECP measurements to disease severity (fig 1, table 2). Moreover, serum ECP concentrations were significantly correlated with pulmonary function (table 2, fig 2).

NEUTROPHIL COUNTS AND SERUM MPO

Neutrophil counts in patients with cystic fibrosis (5510 (3450–7540) cells/μl) were increased compared with healthy controls (4501 (3245–6001) cells/μl; $p < 0.01$). Serum MPO concentrations were also increased in the patients compared with the controls (807 (546–1235) *v* 319 (211–401) μg/l; $p < 0.0001$). In patients with *P aeruginosa* infection or acute pulmonary exacerbation MPO concentrations were significantly higher than in non-infected patients (table 1). Not surprisingly, atopy did not influence neutrophil activities (median MPO concentrations 873 *v* 818 μg/l). Moderate correlations were seen between MPO concentrations and disease severity (fig 1, table 2).

LYMPHOCYTE COUNTS AND SERUM SIL-2R

No difference was seen between lymphocyte counts in patients with cystic fibrosis and in controls (median 2520 *v* 2490 cells/μl). However, sIL-2R concentrations were increased in the patients (4452 (3780–6804) *v* 2743 (2016–3318) pg/ml; $p < 0.0001$) (fig 1). In contrast with the measurements of ECP and MPO, sIL-2R concentrations were significantly higher in children less than 4 years of age compared with the patients after 4 years of age (3696 (3276–4158) *v* 2464 (2016–2716) pg/ml; $p < 0.001$). Neither atopy, *P aeruginosa* infection, acute pulmonary exacerbation, nor the clinical status influenced sIL-2R concentrations (tables 1 and 2).

Discussion

Activated neutrophils and eosinophils have been demonstrated to have a deleterious effect on pulmonary tissue in cystic fibrosis.[7 13] Neutrophil and eosinophil products together with chronic bacterial infection lead to progressive pulmonary destruction, finally resulting in respiratory failure and death. The role of the neu-

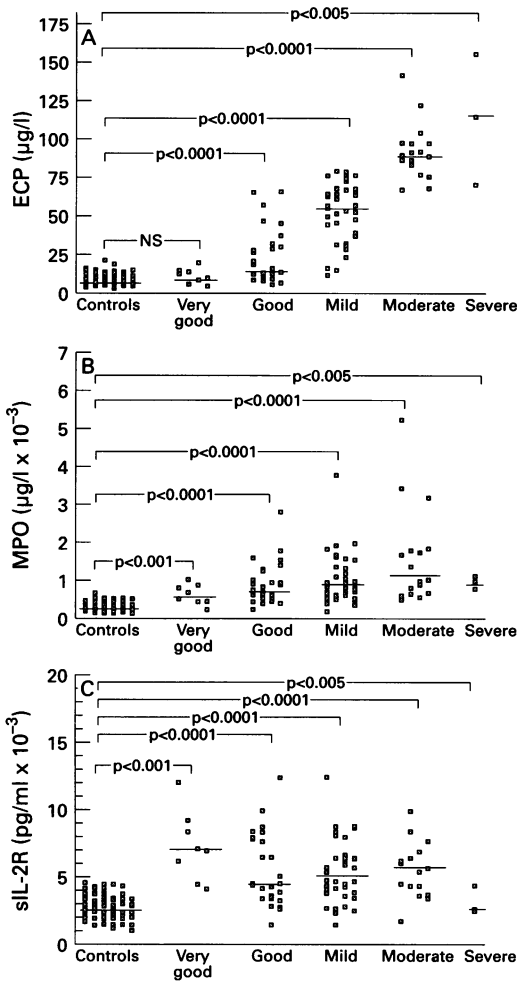

Figure 1 Serum concentrations of (A) ECP in µg/l, (B) MPO in µg/l, and (C) sIL-2R in pg/ml in patients with cystic fibrosis related to the Shwachman-Kulczycki scoring system compared with healthy controls. Median ECP concentrations in patients with cystic fibrosis subdivided into different groups among the Shwachman-Kulczycki scoring system: 1, very good, 11.2 µg/l; 2, good, 16.4 µg/l; 3, mild, 56.3 µg/l; 4, moderate, 89.2 µg/l; 5, severe, 115 µg/l. Median MPO concentrations, 1, very good, 600 µg/l; 2, good, 697 µg/l; 3, mild, 804 µg/l; 4, moderate, 1003 µg/l; and (5) severe, 951 µg/l. Median sIL-2R levels: 1, very good, 6972 pg/ml; 2, good, 4410 pg/ml; 3, mild, 4620 pg/ml; 4, moderate, 5376 pg/ml; and 5, severe, 2562 pg/ml. Statistical differences were calculated by using the Mann-Whitney U test. NS, not significant.

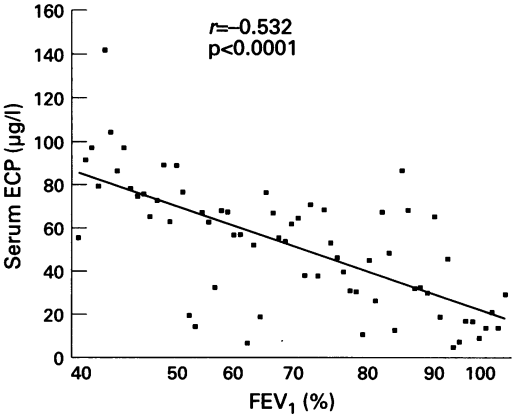

Figure 2 Correlation between serum ECP concentrationss and FEV_1 % predicted in patients with cystic fibrosis. Correlation was calculated by means of the Kendall Tau B test.

trophil and its products in cystic fibrosis were elucidated several years ago.[7] These results show a strong relationship between neutrophil activation—that is, MPO concentrations—and pseudomonas infection or acute pulmonary exacerbation. As acute pulmonary exacerbation is generally accepted to be an indication of intravenous antibiotic treatment in cystic fibrosis, the assessment of MPO in peripheral blood may be used as an indicator to start antimicrobial treatment and to monitor the efficacy of the treatment.[1] We have previously shown that antimicrobial treatment reduces serum MPO concentrations which, however, do not return to normal values in patients with cystic fibrosis.[1] This observation thus allows us to hypothesise that, in addition to antibiotic treatment, anti-inflammatory treatment may be indicated in cystic fibrosis.

Raised sIL-2R serum concentrations also indicate anti-inflammatory efforts. In contrast with MPO or ECP concentrations, we did not observe a relationship between sIL-2R and clinical variables. It appears that sIL-2R concentrations show lymphocyte activation and thus explain the excessive immune response in cystic fibrosis.[6] It has been speculated that lymphocyte activity may be an early indicator of a developing inflammatory process in cystic fibrosis and the first sign of airway infection[3]; however, the role of increased sIL-2R concentrations remains to be further investigated.

The role of the activated eosinophil and its products in cystic fibrosis has been investigated previously.[1 2] The eosinophil is increasingly thought to be a proinflammatory cell in chronic inflammatory respiratory disorders with tissue damaging capacities.[5 13] In cystic fibrosis other mechanisms of eosinophil activation than in bronchial asthma should be considered, as in cystic fibrosis eosinophil numbers are within the normal range.[1] We have shown that eosinophils of patients with cystic fibrosis have an increased propensity to release their granule proteins,[2] which may explain the high ECP concentrations in sputum and serum.[1] Eosinophil activity expressed as ECP concentrations was more related to clinical variables such as pulmonary function and the Shwachman-Kulczycki score than were markers of neutrophils and lymphocytes. It has also been shown that mucociliary clearance is decreased in patients with cystic fibrosis who have a normal lung function.[14] It has been suggested that eosinophil products might be responsible for this phenomenon.[15] We were previously able to show that antipseudomonal treatment did not reduce ECP concentrations.[1] These data support the therapeutic recommendation of the use of anti-inflammatory drugs in cystic fibrosis.[16 17]

In conclusion, the exaggerated inflammatory process in the lungs of patients with cystic fibrosis could be measured in peripheral blood. The assessment of neutrophil activity, measured as MPO concentrations, may be useful in documenting acute pulmonary exacerbations and the infectious status of the patient and to monitor the efficacy of antimicrobial treat-

ment. Concentrations of ECP, a specific marker of eosinophil activation, are strongly related to disease severity in patients with cystic fibrosis and may thus be useful for clinical monitoring in the disease.

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