

Eosinophil cationic protein, myeloperoxidase and tryptase in children with asthma and atopic dermatitis

Kristjánsson S, Shimizu T, Strannegård IL, Wennergren G. Eosinophil cationic protein, myeloperoxidase and tryptase in children with asthma and atopic dermatitis.

Pediatr Allergy Immunol 1994; 5: 223–229. © Munksgaard 1993.

Serum levels of eosinophil cationic protein (ECP), myeloperoxidase (MPO), tryptase, total IgE and differential blood cell counts were studied in atopic children with: 1) moderate to severe asthma using inhaled steroids and symptom-free for the last 3 weeks ($n=13$), 2) mild asthma with sporadic symptoms, using only inhaled β_2 -agonists <3 times/week ($n=15$), 3) acute asthmatic attacks admitted to hospital ($n=12$), 4) mild to moderate atopic dermatitis ($n=14$). Fifteen children without any history of atopy served as controls. ECP, MPO, tryptase and IgE were measured in serum by radioimmunoassays (RIA). The symptom-free children with inhaled steroids had similar median ECP and MPO values as the controls, 8.0 and 360 $\mu\text{g/l}$, vs. 9.0 and 310 $\mu\text{g/l}$, while both ECP and MPO were significantly ($p<0.001$) increased in the symptom-free children without anti-inflammatory treatment, 32 and 887 $\mu\text{g/l}$ and in those with acute asthma, 28 and 860 $\mu\text{g/l}$. The children with atopic dermatitis had increased ECP but normal MPO levels, 16.0 and 455 $\mu\text{g/l}$. Tryptase in serum was not measurable in any patient. All groups except the control group had significantly elevated total IgE levels. The results indicate that in atopic children serum ECP is a good marker of ongoing asthma or atopic dermatitis. The normal levels of ECP and MPO in the children with asthma using inhaled steroids seem to reflect successful anti-inflammatory treatment. The increased levels of ECP and MPO in the children with mild asthma and no anti-inflammatory treatment may indirectly reflect airway inflammation.

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Key words: asthma; atopic dermatitis; atopy; airway inflammation; ECP; MPO; tryptase; β_2 -agonists; corticosteroids.

Received June 21, 1993

Accepted October 21, 1993

The importance of airway inflammation in the pathogenesis of asthma is clearly established (1). Activated eosinophils play an important role in the late asthmatic response and in the asthmatic airway inflammation (2). The eosinophils secrete several toxic, granulae-derived basic proteins. Release of these proteins is dependent on activation of the eosinophils by different cytokines for example IL-5 (3, 4). Activation *in vitro* is also possible with specific allergens or anti-immunoglobulin monoclonal antibodies (5). The most cytotoxic of the proteins are the major basic protein (MBP) and the eosinophil cationic protein (ECP) (6, 7) which, in animal experiments, have been shown to cause airway epithelial damage (8). A correlation has been demonstrated between the severity of asthma and ECP levels in bronchoalveolar lavage (9, 10).

The recognition of the importance of the inflammatory process in the pathogenesis of asthma has changed the pharmacotherapy of asthma largely from that of bronchodilatation to anti-inflammatory treatment. An important question is whether levels of inflammatory mediators like ECP and myeloperoxidase (MPO) may be used to monitor the efficacy of the clinical treatment. ECP and MPO are considered to be specific markers for eosinophils and neutrophils, respectively (11). Although the knowledge of airway pathology in childhood asthma is mainly based on investigations in patients with severe asthma, current treatment guidelines recommend anti-inflammatory treatment to be applied already in children with mild-moderate asthma (12). More information is needed regarding to what extent a measurable inflammatory process exists in the bronchial

airways of largely symptom-free children with mild asthma, who are only using β_2 -agonists.

Patients with atopic dermatitis (AD) frequently have eosinophilia and cytotoxic proteins can be found in skin lesions in patients with AD (13). A correlation has been reported between disease activity in AD and serum ECP level (14).

This study was performed in an attempt to elucidate to what extent ECP, MPO and tryptase in serum and differential blood cell counts may be useful as markers in atopic children with asthma and AD, and to investigate if these markers indirectly may reflect airway inflammation and effects of anti-inflammatory treatment with inhaled steroids.

Subjects and methods

Sixty-nine children were included in the study. Fifty-four had evident atopic disease (40 with asthma and 14 with atopic dermatitis). Fifteen children served as controls. The study was performed outside the pollen season between September 1991 and March 1992 and between October 1992 and February 1993. Before inclusion parental approval was given and a detailed medical history was obtained. Determination of asthma severity was based on frequency of asthmatic symptoms and need of treatment to remain symptom-free (12). AD was defined according to Hanifin (15) and a modified clinical scale (1–10 points) was used to determine the severity of AD. All subjects included were examined by the same doctor (SK) and a protocol was completed on the severity of asthma or AD. The 40 patients with asthma were divided into three different groups according to the severity of asthma and the treatment:

1) patients with moderate to severe asthma using inhaled steroids continuously (daily dose 400 μ g) for a longer time than 2 months and being symptom-free for the last 3 weeks, $n = 13$; median age 11.8 (range 4.7–15.7) years; 2) patients with mild asthma with

sporadic symptoms, only using inhaled β_2 -agonists <3 times/week, but symptom-free when included, $n = 15$; 10.3 (1.5–16) years; 3) patients with acute asthmatic attacks admitted to hospital, $n = 12$; 6.8 (2.0–14.6) years. The children with mild or moderate AD at the time of investigation formed a fourth group, $n = 14$; 5.7 (0.2–14) years. Children without atopic disease or any other known disease served as controls, $n = 15$; age 10.4 (0.7–16) years.

Subject characteristics

The characteristics of the groups with regard to age, peak expiratory flow value (PEF) and treatment are shown in Table 1. There were no statistically significant differences in age between the groups. Atopy was defined as positive skin prick test or positive S-RAST to one or more common allergens. All subjects except the controls and one child with AD fulfilled these criteria of atopy. No patient had signs of urticaria or allergic rhinoconjunctivitis when examined.

All the patients in Groups 1 and 2 (i.e. subjects with moderate-severe and mild asthma, respectively) were subjectively healthy and on direct question they denied having any current asthmatic symptoms. They had no signs of infection and physical examination revealed no rhonchi or other signs of asthma. PEF was measured using a Wright Peak Flow Meter. The PEF results were compared to a reference material and expressed as standard deviations (S.D.) of the mean value for the patient's height (16). The patients with acute asthma were all suffering from asthmatic symptoms that had lasted between 1–20 days. All these patients were hospitalised because of asthma and treatment with bronchodilators and steroids, 2–300 mg hydrocortisone (Soluortef®, Upjohn) i.v. or 6 mg of betamethasone (Betapred®, Glaxo) p.o., had already been started 6–18 h before the blood sampling. Most of the children with acute asthma had signs of respiratory tract

Table 1. Subject characteristics. All patients, except the controls had asthma or atopic dermatitis as their major atopic disease. Peak expiratory flow rate, PEF (l/min), is expressed as standard deviations from the mean value for the patient's height (16). Ranges are given within brackets.

Groups	N	Median age, years	Median PEF, S.D.	Daily dose of inhaled steroids, mg		Treatm. duration, months
				Inhaled budesonide ¹	Inhaled beclometh. ²	
Controls	15	10.4 (0.7–16)	–	0	0	
Asthma, inhaled steroids, symptom free	13	11.8 (4.7–15.7)	0.0 (–0.2–1.5)	0.4 (0.4)	0.4 (0.4)	6.0 ¹ ; 24.0 ² (2–9) ¹ ; (6–84) ²
Asthma, β_2 -agonists only, <3 times/week	15	10.3 (1.5–16)	–0.5 (–2.0–1.5)	0	0	
Acute asthma	12	6.8 (2–14.6)	–	–	–	
Atopic dermatitis	14	5.7 (0.2–14)	–	0	0	

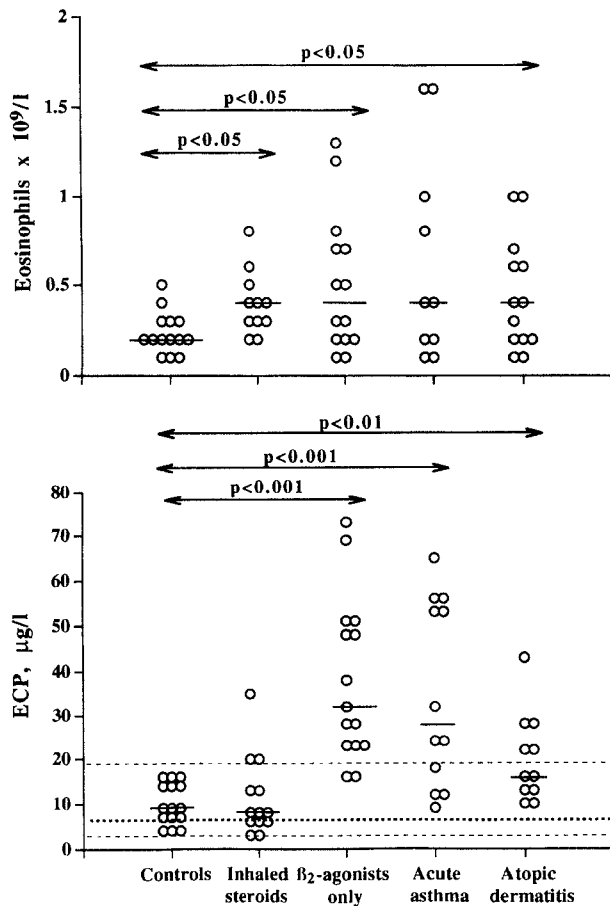


Fig. 1. Serum ECP levels (lower part) and eosinophil values (upper part). Median values and significant differences between patient groups and controls are shown. Non-parametric Mann-Whitney U-test was used for statistical comparison. Mean, and upper and lower 100% range for ECP in 101 healthy adults are indicated (11, 17).

infection of viral origin. The children with AD had a mild or moderate ongoing disease, i.e. $\leq 25\%$ of the body surface area involved, and no symptoms or signs of other allergic disease when examined.

Blood analyses

Venous blood for investigation was sampled after application of local anaesthesia with lidocain ointment (EMLA®, Astra). Blood for measurement of ECP, MPO and tryptase was collected in Vacutainer® SST tubes and converted 5 times after collection. The blood was allowed to clot at room temperature for 60 min and thereafter serum was separated by centrifugation, twice for 10 min at 1200 g and then transferred to a new test tube and stored at -20°C until the analysis was performed.

ECP, MPO and tryptase were analysed using commercial radioimmunoassay (RIA) kits (Kabi Pharmacia Diagnostics). ECP and MPO were measured in serum by a double-antibody RIA, which is a competitive assay. Tryptase in serum was measured

by a sandwich assay. The reproducibilities of the assays are good, inter- and intra-assay coefficients of variation being less than 10% for all these tests. The detection limit of the tests was 2 $\mu\text{g/l}$ in all cases (11, 17). Total IgE was measured by a RIA assay (Kabi Pharmacia Diagnostics).

For absolute leukocyte and differential counts a cytochemical analysis with Technicon H® system was performed. Blood was drawn by venipuncture and collected in a vacutainer tube with EDTA.

In an attempt to relate the serum levels of ECP and MPO to cell counts, the ratios ECP ($\mu\text{g/l}$) : eosinophils $\times 10^9/\text{l}$, and MPO ($\mu\text{g/l}$) : neutrophils $\times 10^9/\text{l}$, were calculated.

Statistics

Statistical analyses were performed using the Stat-View SE + Graphics™ software package. Comparisons between groups were made using the non-parametric Mann-Whitney U-test. P-values < 0.05 were considered statistically significant. The study was approved by the Ethics Committee of the Medical Faculty, University of Gothenburg.

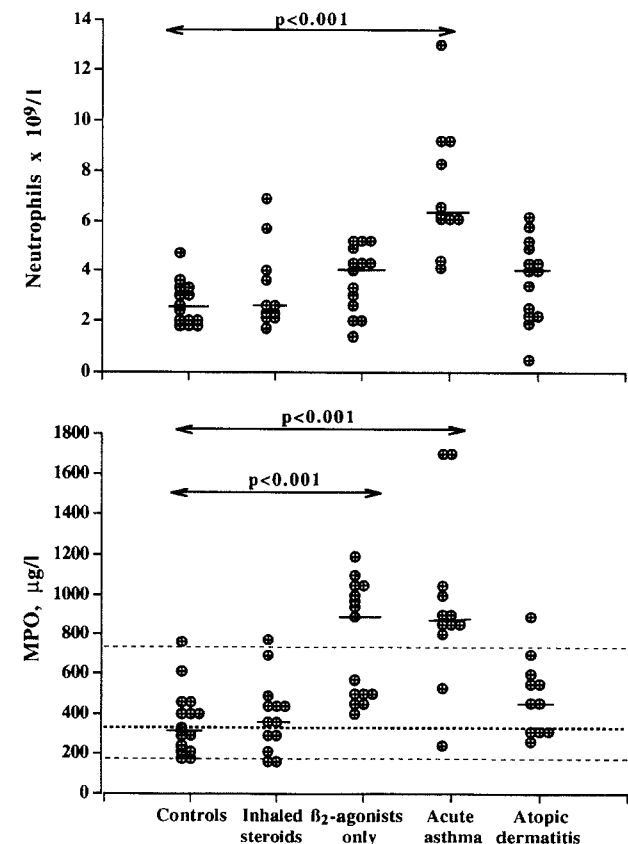


Fig. 2. Serum MPO levels (lower part) and neutrophil values (upper part). Median values and significant differences between patient groups and controls are shown. Non-parametric Mann-Whitney U-test was used for statistical comparison. Mean, and upper and lower 100% range for MPO in 101 healthy adults are indicated (11, 17).

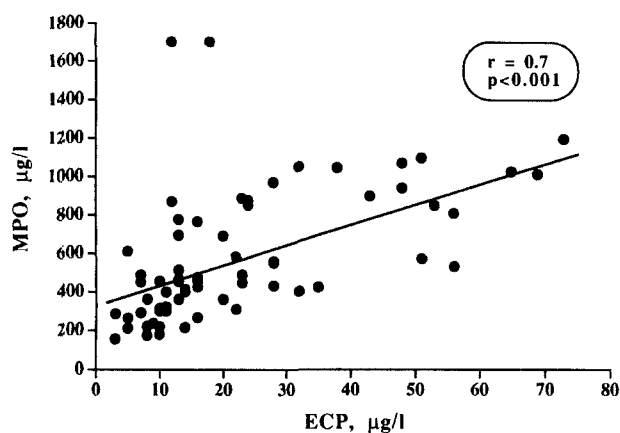


Fig. 3. Correlation between ECP and MPO in serum. Data from all patients and controls.

Results

ECP, MPO and tryptase

The median S-ECP level was 9.0 (range 4.0–16) µg/l in the control group (Fig. 1). The patients with moderate-severe asthma treated with regularly inhaled steroids had similar levels, 8.0 (2–35) µg/l. One patient had a ECP value of 35.0 µg/l. He had a cat at home and a positive RAST class 3 against cat allergen. By contrast, the ECP levels were significantly ($p < 0.001$) higher in the children with mild asthma, but no anti-inflammatory treatment, only using β_2 -agonists, 32.0 (12–73) µg/l. Only two of these patients had an ECP value within the normal range. The children with acute asthma also had significantly increased levels of ECP, 28 (9–65) µg/l. However, almost all of these children had received one or more doses of steroids i.v. or p.o. 6–18 h before the blood was drawn. Increased S-ECP levels were also seen in the group of children with AD, 16 (10–43) µg/l, but 50% of this group had ECP levels within the normal range.

Serum MPO levels (Fig. 2) were similar in the controls, 310 (175–764) µg/l, in the steroid-treated group, 360 (155–776) µg/l and in the children with AD, 455 (265–898) µg/l. By contrast, the asthmatic children treated only with β_2 -agonists as well as the children in the 'acute asthma' group had significantly increased MPO levels, 887 (403–1198) and 860 (236–1700) µg/l.

The ECP and MPO levels were significantly higher in the group of children using ' β_2 -agonists only' than in the 'inhaled steroids' group ($p < 0.001$). Tryptase was not detectable in serum from any asthmatic children, children with AD or controls.

Leukocytes and total IgE

Leukocyte, as well as neutrophil, counts were significantly increased in the 'acute asthma' group compared to the controls (Table 2, Fig. 2). The neutrophil count in the 'acute asthma' group was significantly higher also compared to the group of children with asthma using ' β_2 -agonists only' ($p < 0.001$). The eosinophil count (Fig. 1, Table 2) was significantly increased in all patient groups except the 'acute asthma' group. Levels of total IgE were significantly increased in all patient groups compared to the control children (Table 2).

Correlations and ratios

A significant correlation ($r = 0.7$; $p < 0.001$) was found between ECP and MPO levels (Fig. 3). The MPO per neutrophils $\times 10^9$ ratio was significantly increased ($p < 0.05$) in the group with mild asthma and no anti-inflammatory treatment (compared to controls), while the ECP per eosinophils $\times 10^9$ ratio was not ($p = 0.08$) (Table 2). The correlation between increased S-MPO and S-ECP > 20 µg/l levels, and decreasing PEF were $r = -0.6$ ($p = 0.06$) and $r = -0.5$ ($p = 0.09$), respectively.

Table 2. Leukocyte and total IgE values in controls and patients. The serum levels of ECP and MPO have been related to the eosinophil and neutrophil cell counts, respectively. Results are given as median values with ranges in parenthesis. Statistically significant difference versus controls is indicated by stars ($p < 0.05 = *$; $p < 0.01 = **$; $p < 0.001 = ***$). Non-parametric Mann-Whitney U-test was used.

	Controls	Inhaled steroids	β_2 -agonists only	Acute asthma	Atopic dermatitis
Leukocytes $\times 10^9/l$	5.5 (4.0–12.1)	7.1 (4.3–9.8)	6.9 (5.1–8.8)	9.8** (7.0–15.1)	7.5 (4.7–10.9)
Eosinophils $\times 10^9/l$	0.2 (0.1–0.5)	0.4* (0.1–0.8)	0.4* (0.1–1.3)	0.4 (0.1–1.6)	0.4* (0.1–2.7)
ECP/EOS: µg/ 10^9 cells	50 (23–160)	33 (5–97)	87 (24–530)	78 (9–400)	56 (10–144)
Neutrophils $\times 10^9/l$	2.7 (1.9–4.7)	2.6 (1.7–6.9)	3.8 (1.4–5.3)	6.4*** (4.1–13)	4.0 (0.5–6.2)
MPO/Neut: µg/ 10^9 cells	160 (37–283)	105 (55–248)	196* (87–686)	128 (55–284)	144 (63–239)
Total IgE, kU/l	23 (2–470)	380*** (80–1700)	590*** (17–3300)	320** (14–6500)	500* (2–5800)

Discussion

The pathophysiology of asthma is not fully known but inflammation in the airways is one of the main pathological findings in severe asthma. The airway inflammation probably begins with specific or unspecific stimulation of the bronchial epithelial cells. This leads to activation of mast cells, lymphocytes, eosinophils and probably neutrophils. The release of inflammatory mediators such as cytokines and leukotrienes from the activated cells play important roles in the pathogenesis. At least two of the eosinophil-derived proteins, MBP and ECP, can damage the respiratory epithelium through their cytotoxic effects (8). The epithelial damage may lead to loss of the permeability barrier and changed composition of the airway fluid lining. One of the end results is the development of a hyaline band beneath the epithelium and later, with increased severity of the disease, chronic, irreversible changes in the airways are established (18–21). In addition to being markers of disease activity, products derived from eosinophils may thus play a pathogenetic role in asthma. Bousquet et al (10) recently found increased levels of ECP in bronchoalveolar lavage (BAL) fluid of adult asthmatics with moderate or severe asthma. They demonstrated a correlation between the ECP levels in BAL and the severity of the asthma in patients without anti-inflammatory treatment. Ädelroth et al (22) found increased ECP levels in serum and BAL fluid of asthmatic patients inhaling β_2 -agonists and steroids irregularly, and S-ECP and BAL levels of ECP showed a significant correlation.

The pathogenesis of atopic dermatitis is also insufficiently known, but patients with AD frequently have eosinophilia and increased IgE levels. Eosinophils are thought to be important as effector cells mediating late-phase allergic reaction in the skin tissue, and cytotoxic eosinophil-derived basic proteins can be found in skin lesions of these patients (13–14, 23).

In the present study the serum levels of ECP and MPO were significantly increased in children with mild asthma who were using β_2 -agonists only and in children hospitalised because of an asthmatic attack. By contrast, ECP and MPO levels were found to be normal in children with moderate to severe asthma who had been treated with inhaled steroids on a regular basis for more than two months and had been symptom-free for the last 3 weeks. The fact that the children with 'acute asthma' had already received their initial dose of steroids before the blood sample was drawn, most likely explains why their median ECP value was not higher than in the ' β_2 -agonists only' group. The half life of ECP is estimated to be about 65 min (17).

S-ECP was significantly increased also in the children with AD, while mean MPO was normal. In adults with moderate to severe AD, ECP has been shown to be increased and to correlate with disease activity (14). In the present paper, the children studied had mild-moderate AD which may be one reason for their relatively moderate increase in mean ECP level. The rapid turnover of the mast cell marker tryptase (11) probably explains why we were unable to detect it in serum from any of the studied subjects.

Reports on the role of neutrophils in the asthmatic inflammation are contradictory. Studies based on histological findings, immunofluorescence technique or lung lavage in asthmatic subjects or using animal models suggest that neutrophils are involved in the asthmatic reaction (24–26), where the neutrophils seem to be involved initially in the late asthmatic reaction and thereafter replaced by eosinophils (24). Schmekel & Venge (27) found that substantial amounts of MPO are deposited in the mucosa of the proximal airways also in healthy subjects. Carlson et al (28) have reported that birch pollen allergic subjects with asthma have increased secretion of MPO from isolated neutrophils during the pollen season. By contrast, Bousquet et al (10) were unable to find increased levels of MPO in BAL fluid of patients with asthma, which supports the results of a study of bronchial biopsies which failed to reveal any increase in the number of neutrophils in the bronchi of asthmatic subjects (29).

In our study MPO as well as the MPO/neutrophil ratio was significantly increased in the group with mild asthma using inhaled β_2 -agonists but having no anti-inflammatory treatment. This finding indirectly may indicate a more active role of the neutrophils in the asthma process than commonly recognised, since these patients did not have signs of an ongoing infection, and their neutrophil count was normal. Earlier studies have failed to demonstrate increased MPO levels in patients with asthma, although Carlson et al have found increased release of MPO from neutrophils in asthmatic patients (30). Another question that can be raised is whether the increase in MPO could be an effect mediated by β_2 -agonist cell stimulation.

In the group with moderate-severe asthma, regularly treated with inhaled steroids, the mean ECP as well as MPO levels were normal. Likewise their mean PEF values were normal (Table 1). These findings are in accordance with those of Ädelroth et al (22) regarding ECP. They found normal mean ECP levels in serum and BAL fluid in asthmatic patients inhaling steroids regularly.

In the children with mild asthma only inhaling β_2 -agonists but having no anti-inflammatory treatment, there was a trend to increasing MPO and ECP levels being correlated to decreasing PEF values. To

make reliable conclusions about correlation between these markers and lung function, a prospective longitudinal study where detailed lung function tests are included is necessary.

We found a significant overall correlation between the ECP levels and eosinophil counts in blood, and between MPO levels and neutrophil counts. However, the group with regular steroid treatment had normal ECP levels but significantly increased levels of eosinophils, while the increased MPO levels in the ' β_2 -agonist' group were not associated with increased neutrophil counts. These examples from individual patient groups show that the serum levels of proteins derived from eosinophils and neutrophils do not necessarily correlate with the corresponding cell counts. This is of importance when considering the future clinical value of measurements of these markers.

An objective monitoring of therapy is very important in patients with asthma, who often show a poor drug compliance, in particular regarding anti-inflammatory treatment (31). Both ECP and MPO could prove to be very useful for the monitoring of anti-inflammatory effects in atopic patients with asthma.

In conclusion, in children with mild and moderate AD, S-MPO was not elevated, while ECP was. ECP can probably be used as a marker of effective anti-inflammatory treatment with inhaled steroids in children with asthma. MPO in serum as well as ECP were increased in children with mild asthma and without anti-inflammatory treatment. These findings may indirectly indicate airway inflammation and a more important role of the neutrophils in the pathogenesis of asthma than commonly recognised. In atopic children serum ECP seems to be a good marker of ongoing asthma or atopic dermatitis.

Acknowledgements

The authors thank Mrs. Agneta Wyholt, Registered Nurse, for her expert help. This study was supported by grants from the First of May Flower Annual Campaign for Children's Health, the Swedish National Association against Asthma and Allergy, the Swedish Heart Lung Foundation, the Th Bergh Research Foundation, the Gothenburg Masonic Order Orphanage Foundation, and the Research Fund of the Children's Clinics, Gothenburg.

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