

Individual time-courses of ECP and EPX during allergen provocation tests in asthmatic children

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To study the time-course of eosinophil cationic protein (ECP) and eosinophil protein X (EPX) during bronchial allergen provocation, we investigated 32 asthmatic children sensitive to house-dust mites as well as 6 non-atopic young adult controls. In all subjects, allergen challenges were performed with house dust mite extracts of *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*. Blood samples were taken at regular intervals during the 24-h observation period. The individual time-courses of ECP and EPX revealed different characteristic groups of patterns: (1) an isolated early serum peak of both mediators during or within the first 60 min after provocation (2) an early plus a late peak (3) an isolated late peak 12 h after provocation (4) an isolated late peak 24 h after provocation, and (5) no significant variation during the 24-h observation period. The early peak could be due to short-term changes in eosinophil activation, while late peaks may reflect eosinophil proliferation, recruitment, subsequent priming and enhancing of the propensity to release their proteins. ECP and EPX showed a corresponding parallel time-course in nearly all challenges, with EPX-concentration exceeding that of ECP. There was no correlation between ECP/EPX serum concentrations and clinical parameters such as lung function data. From our results we conclude that the striking groups of time-courses of ECP/EPX serum concentration indicate different uniform patterns of eosinophil activation during allergen challenge – but do not predict clinical outcome of provocation. The role of the eosinophil in early asthmatic reactions remains to be established in further studies.

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In recent years the importance of airway inflammation in the pathogenesis of bronchial asthma has become apparent from many experimental and clinical studies. Mechanisms responsible for the ongoing mucosal inflammatory process are still not entirely understood, but a number of studies point to a crucial role for the eosinophil granulocyte (1, 2). Eosinophil accumulation is a characteristic finding in lung tissues obtained from subjects after death from acute asthma (3). Furthermore, it is a common finding in asthmatic patients during day-to-day asthma

(4). In addition, several studies have demonstrated a relationship between the extent of blood eosinophilia and the severity asthma (5). Eosinophils are considered to be proinflammatory cells, especially because of their capacity to secrete several highly cytotoxic proteins (ECP, EPX, EPO, MBP) (6–8). Evidence of increased secretion of eosinophil granule proteins in asthmatic patients is known from various clinical studies (9, 10). ECP and MBP have been shown to cause airway epithelium damage resembling the histopathological findings in asthma (11, 12).

To study the late asthmatic reaction (LAR) to bronchial allergen challenge provides a model for investigating the inflammatory mechanisms in asthmatic patients. In bronchoalveolar lavage (BAL) of adult asthmatic patients during the LAR after allergen provocation, some authors found an eosinophilia (13, 14). Furthermore, an elevation of activated eosinophil levels could be demonstrated in

ECP: eosinophil cationic protein, EPX: eosinophil protein X, Dpt: dermatophagoides pteronyssinus, Df: dermatophagoides farinae, EAR: early asthmatic reaction, LAR: late asthmatic reaction, DAR: dual asthmatic reaction, NAR: no asthmatic reaction, FEV₁: forced expiratory volume in one second, PC₂₀ FEV₁: provocation concentration of Dpt or Df which induces a 20% fall of FEV₁, PEF: peak expiratory flow, BAL: bronchoalveolar lavage, SQU: standard quantity unit.

Table 1. Association between early/late serum ECP/EPX peak and type of clinical reaction in 32 asthmatic children

Group	Pattern	n	EAR	DAR	LAR	NAR
1	Isolated early peak	7	1	5	0	1
2	Early plus late peak	11	1	9	1	0
3	Late peak at 12 h	5	1	2	2	0
4	Late peak at 24 h	5	2	2	1	0
5	No peak	4	0	2	1	1
	n	32	5	20	5	2

BAL, which was followed by elevated ECP BAL-levels at 24 h after challenge (15).

In terms of investigation of eosinophil granule proteins in peripheral blood before and during allergen bronchial provocation, several studies have been performed since the first investigation by Dahl et al. 1978 (5, 16, 17). However, only Dahl et al. measured serum-ECP at close time intervals during the early asthmatic reaction (EAR) and up to 24 h after allergen challenge (16).

In a recent study we investigated the time-course of ECP and EPX serum concentration in 32 asthmatic children and 6 non-atopic controls before and during allergen bronchial challenge (18). In that study we could not confirm that serum concentration of ECP and EPX are useful parameters for an individual monitoring of clinical reactions to bronchial allergen challenges. However, evaluation of the individual time-courses revealed a clustering of uniform patterns of ECP and EPX serum concentration which are presented and discussed in this paper.

Material and methods

We investigated 32 children (26 boys, 6 girls) aged 9 to 17 years (mean 12 years) and 6 non-atopic young adults. All children suffered from mild to moderate bronchial asthma, as defined by the American Thoracic Society (19). Baseline FEV₁ ranged from 65% to 120% predicted (mean 93%). House-dust mite sensitivity was the predominant sensitivity of these children and was verified by detection of specific IgE-antibodies against *Dermatophagoides pteronyssinus* (Dpt) and *Dermatophagoides farinae* (Df) (PCS-class ≥ 2 , mean 4.5).

Six non-atopic young adults (aged 24 to 43 years, mean 28 years) served as controls (total IgE < 100 kU/l). No specific IgE-antibodies were found against Dpt or Df. Baseline FEV₁ ranged from 85% to 118% predicted (mean 95%). All patients and their parents, as well as the controls, gave their verbal consent before starting the investigations.

Study design

Children and controls were challenged with Dpt or Df allergen extract. All challenges started at 0900 in order to avoid circadian influences (20). Patients and controls were followed for 24 h in a hospital setting. During the procedure, peak-flow was measured at hourly intervals up to 12 h, as well as at 24 h after provocation. Blood samples were taken at regular intervals during the observation period.

An early peak of mediator serum concentration (ECP and/or EPX) was defined as an increase of at least 100% from the mean individual baseline value and/or an 100% decrease thereafter; a late peak at 12 h or 24 h was defined as an increase of at least 100% from the mean individual baseline value at these time-points.

Methods

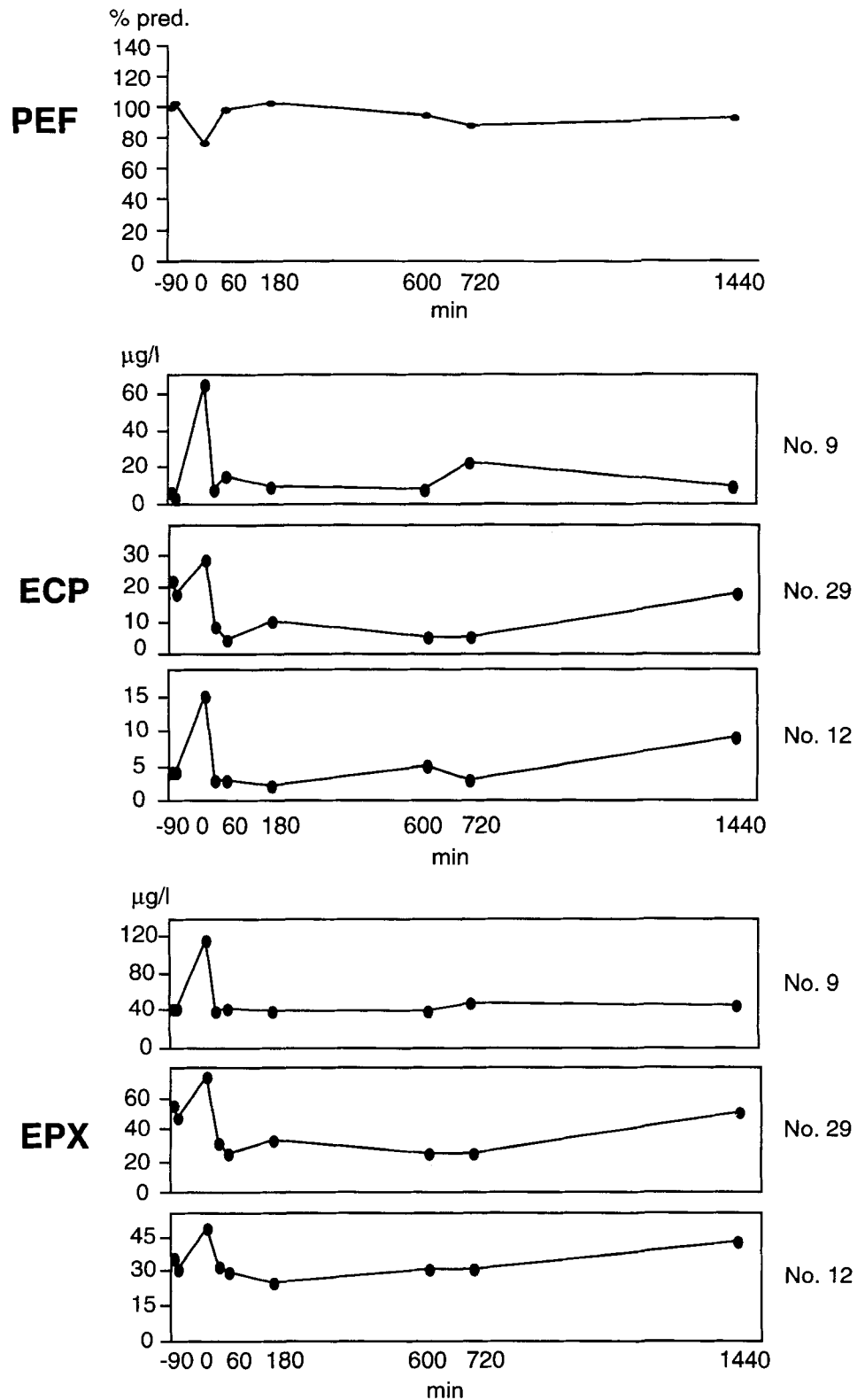
Lung function tests were performed using a bodyplethysmograph (E. Jaeger, Würzburg, Germany). After baseline lung function (inhalation of the diluent), titrated provocation was undertaken with a jet nebulizer (Pari-boy, Medanz, Starnberg, Germany). Each concentration was inhaled for 5 min.

Lung-function at baseline, at time of maximal response and after inhalation of a bronchodilator were performed as a full bodyplethysmographic measurement. Directly and 5 min after each concentration during the provocation procedure, FEV₁ was measured by forced spirometry maneuver (flow-volume-curve) using the pneumotachograph of the bodyplethysmograph. In order to achieve comparable peak-flow-values over the 24-h time-period, peak-flow was measured during the provocation procedure using a mini-Wright peak-flow-meter in addition to the PEF obtained by flow-volume-curve. Three peak-flow measurements were performed, the best value was noted, a variation up to 10% was tolerated. PC₂₀ FEV₁ determined the early asthmatic reaction, while 24-h peak-flow protocol detected a possible late asthmatic reaction. Biological standardized extracts of Dpt or Df (ALK, Copenhagen, Denmark) in concentrations ranging from 10 to 100 000 SQU were used.

Blood samples were drawn from an antecubital venous catheter at 9 time points (2 baseline values within 10 min before provocation, at 0, 30, 60, 180, 600, 720 and 1440 min after provocation). Serum was prepared according to guidelines recently published (21), and was stored at -20°C until analyzed.

Eosinophil granule proteins (ECP, EPX) were determined from serum by radioimmunoassay (Pharmacia, Uppsala, Sweden) (10, 21, 22). All assays were run in duplicate. The coefficient of intra-assay variation was 7.9% for ECP and 4.3% for EPX; the

Fig. 1. Three individual time-courses of serum ECP- and EPX- concentrations and the mean peak-flow pattern representing the group of asthmatic children with an isolated early peak (Patients 9, 29, 12).



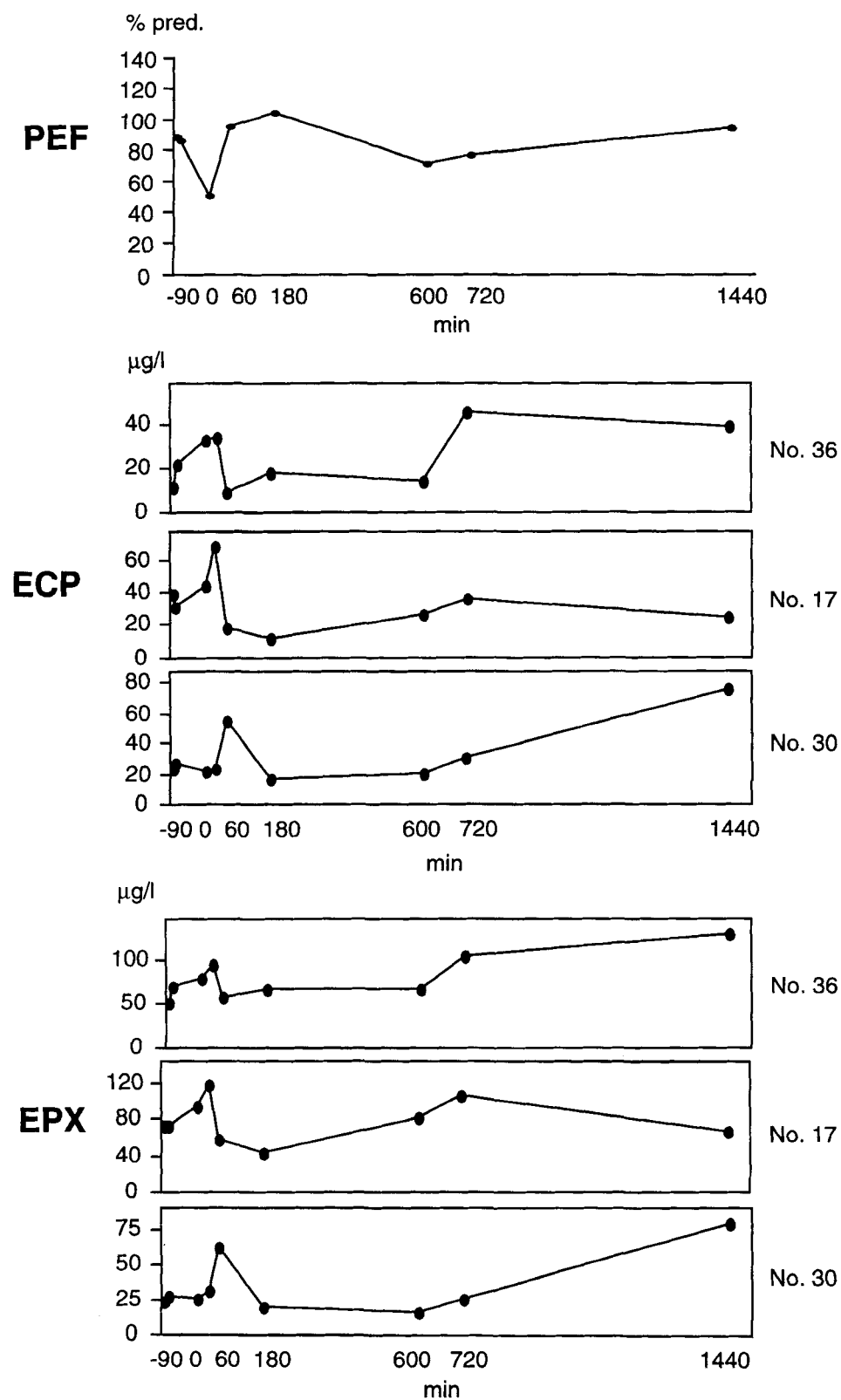
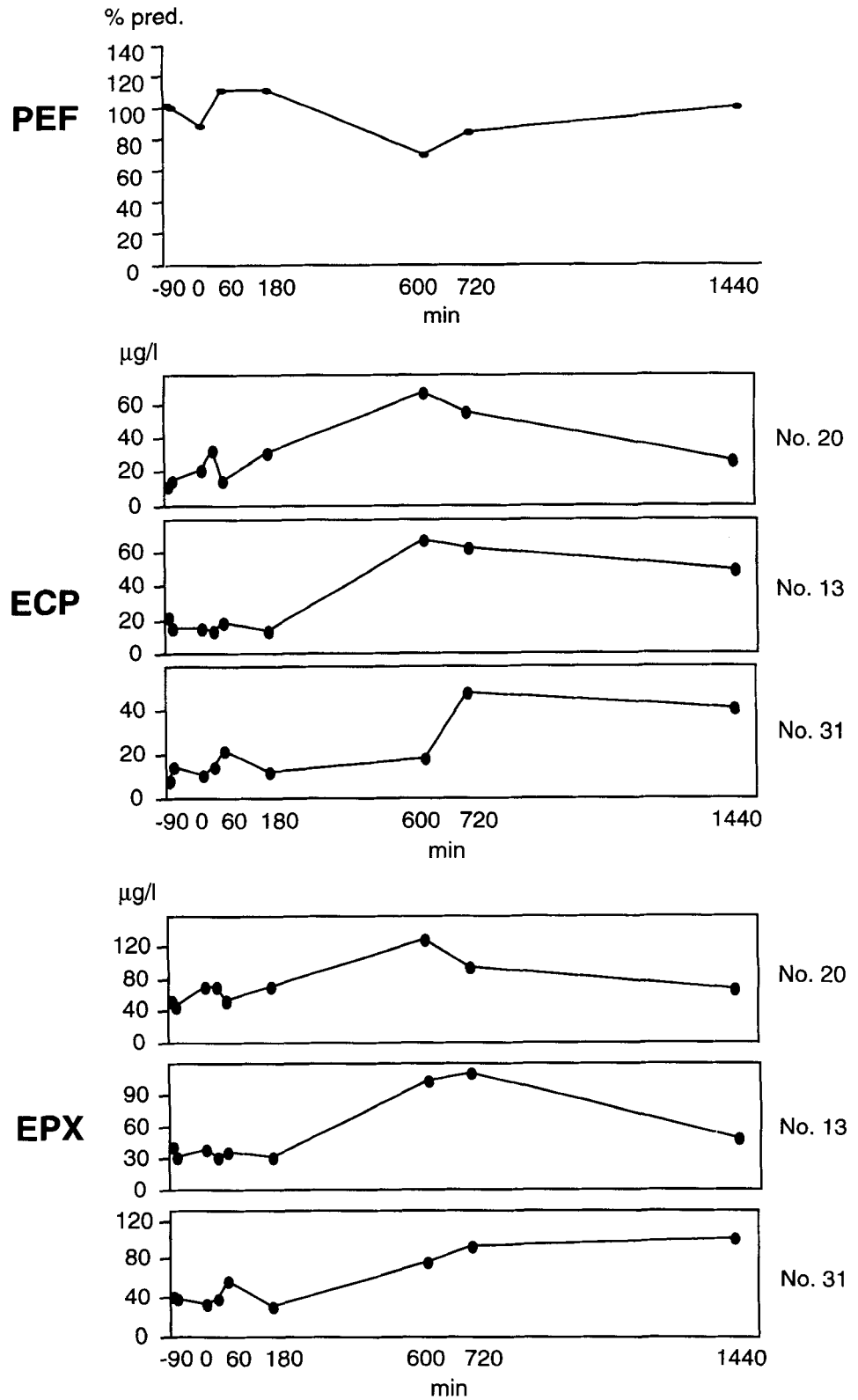


Fig. 2. Three individual time-courses of serum ECP- and EPX-concentrations and the mean peak-flow pattern representing the group of asthmatic children with an early plus late peak (Patients 36, 17, 30).

Fig. 3. Three individual time-courses of serum ECP- and EPX-concentrations and the mean peak-flow pattern representing the group of asthmatic children with an isolated late peak 12 h after bronchial allergen provocation (Patients 20, 13, 31).



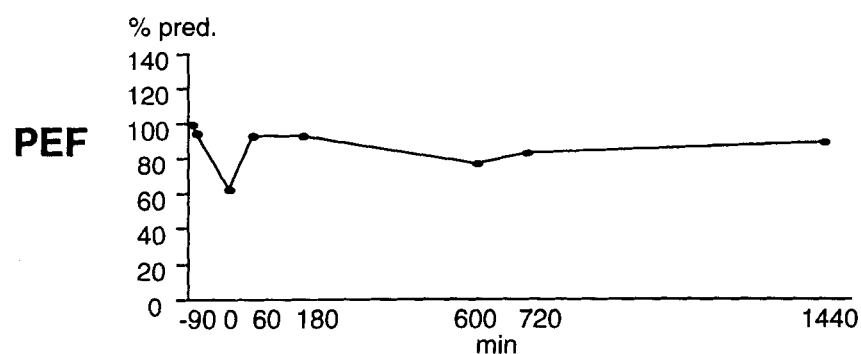


Fig. 4. Three individual time-courses of serum ECP- and EPX-concentrations and the mean peak-flow pattern representing the group of asthmatic children with an isolated late peak 24 h after bronchial allergen provocation (Patients 19, 16, 28).

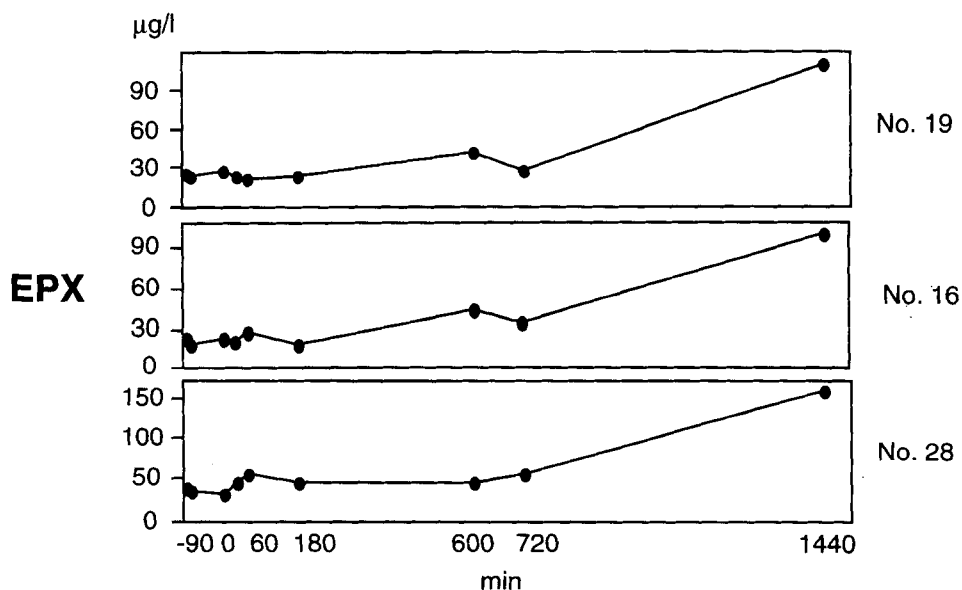
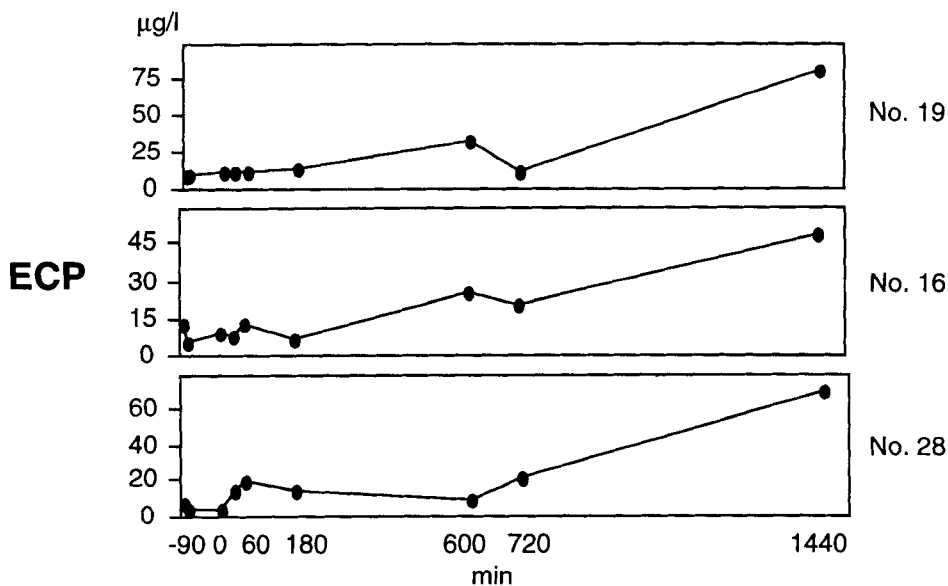
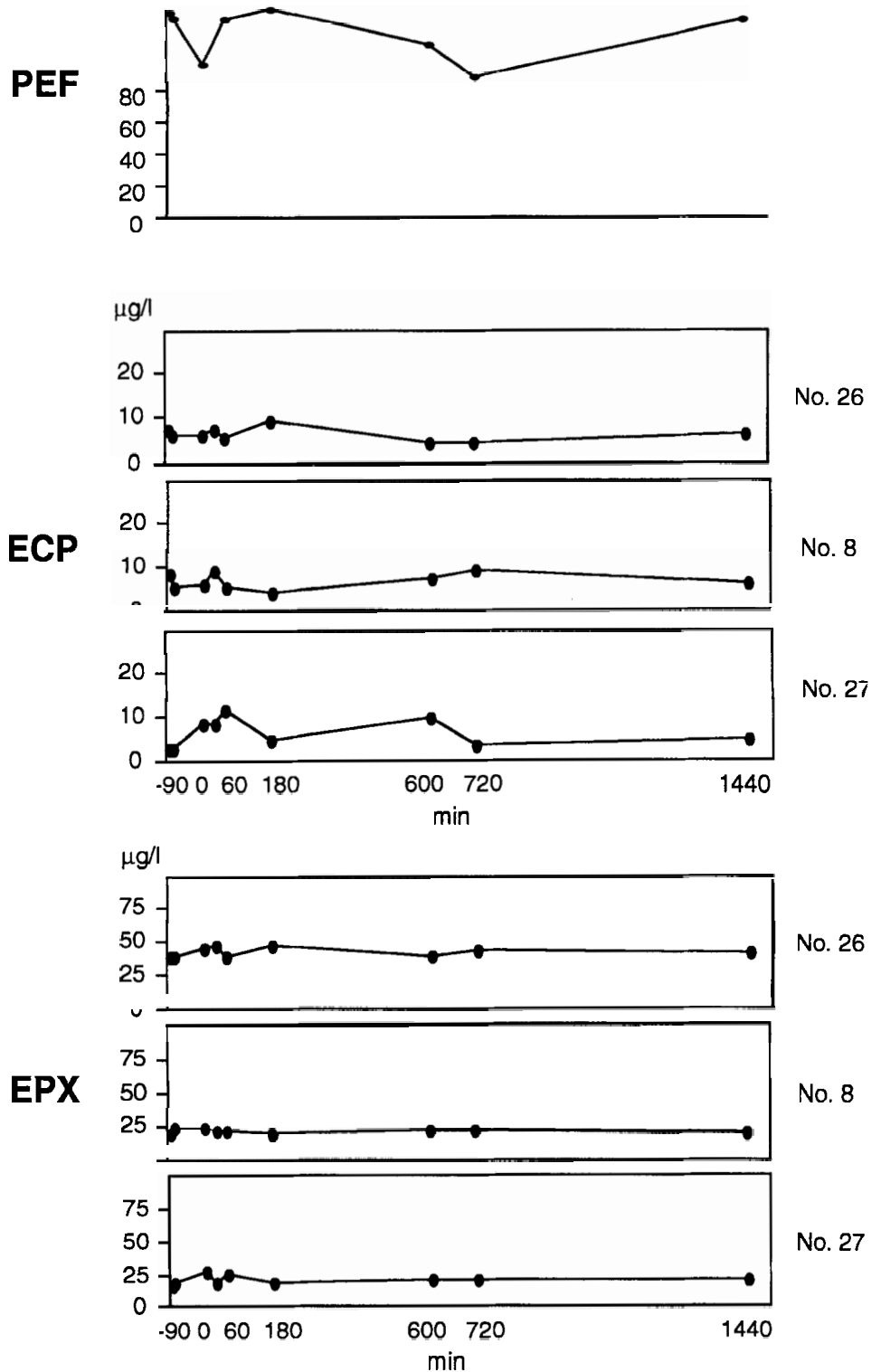


Fig. 5. Three individual time-courses of serum ECP- and EPX-concentrations and the mean peak-flow pattern representing the group of asthmatic children with no significant variation (Patients 26, 8, 27).



inter-assay coefficient of variation was 4.7% for ECP and 6.8% for EPX. For statistical analysis, Mann-Whitney-U-test, linear rank correlation test and multiple regression analysis were used.

Results

The individual time-courses of serum ECP and EPX concentration in our 32 children revealed five different patterns of response (Table 1): *Group 1* (n = 7): patients with an isolated early serum peak of both mediators during or within the first 60 min after provocation (Fig. 1), *Group 2* (n = 11): patients with an early plus a late peak (Fig. 2), *Group 3* (n = 5): patients with an isolated late peak at 12 h after provocation (Fig. 3), *Group 4* (n = 5): patients with an isolated late peak at 12 h after provocation (Fig. 4), and *Group 5* (n = 4): patients with no variation during the 24-h observation period (Fig. 5). None of the non-atopic controls showed any significant serum ECP/EPX variation.

No correlation between serum concentration of both mediators and the type of clinical reaction (EAR, LAR, DAR and NAR = no asthmatic reaction) could be found by means of a linear rank correlation test and multiple regression analysis. This was valid for the corresponding time points (e.g. early peak and EAR) as well as for any other combination (e.g. early peak and LAR or vice versa).

Comparing serum concentration of both mediators, there was a striking parallel time-course in nearly all challenges, with EPX-concentration exceeding that of ECP in all provocations.

Discussion

There is a lack of information about the variation of serum ECP and EPX concentrations during bronchial allergen challenge in asthmatic patients. We could not confirm a suspected correlation between serum concentration of both mediators and the type of clinical reaction. However, there was a striking clustering of similar patterns considering individual time-courses of serum mediator concentration.

18/32 children showed an early peak for both mediators, irregardless of the occurrence of a subsequent late peak. This early peak during allergen challenge has only been described before by two authors (12, 16). Our hypothesis for this early peak is that the sudden increase and rapid decrease of eosinophil mediator concentrations reflect short-term changes of eosinophil activation. Activation of eosinophils can be induced by various mediators from inflammatory cells involved in early asthmatic reactions (e.g. mast cells and platelets) such as PAF and RANTES (2). Evidence for this hypothesis is provided by studies which have demonstrated that

e.g. IL-5, C5a and RANTES are capable of stimulating intracellular mobilization and activation of eosinophil granule proteins (23). PAF is an inducer of hypodense eosinophils and eosinophil activation (24). In addition, platelets decrease in peripheral blood during allergen-induced early asthmatic reactions (25).

Rapid decrease of both mediators back to baseline values within 30 min is not what one would expect given an elimination period of 65 min (21). One explanation for this phenomenon could be that the measurement in serum (according to the guidelines) shows ECP that is released *in vitro* during clotting time – not ECP present in peripheral blood (26). Therefore, differences in mediator concentration in blood samples taken at short intervals may reflect short-term activation changes of the eosinophils at the time of blood sampling.

A late increase of serum mediator concentration was observed in different patterns: some patients showed a peak at 12 h after provocation, some after 24 h. No precise pathomechanisms responsible for late mediator increases are known. One might hypothesize that allergen provocation in sensitized individuals leads to T-lymphocyte activation, followed by eosinophil proliferation and recruitment from bone marrow as well as subsequent priming e.g. enhancing of the propensity of the eosinophils to release their proteins. This view can be supported by studies which have demonstrated an increase of activated CD4⁺ T-cells in BAL and bronchial biopsies 24 h after allergen challenge (27, 28). Additionally, these studies noted a decrease of CD4⁺ T-cells in peripheral blood, interpreted as a specific recruitment of these cells into the respiratory system (29). Cytokines secreted by activated T-cells such as IL-5 have an important priming effect for human eosinophil degranulation *in vitro* (30) and *in vivo* (31). Activated hypodense eosinophils appear in peripheral blood in antigen-dependent late phase asthmatic reactions (32). Recruitment of activated eosinophils into airways is followed by degranulation of eosinophil derived proteins (15).

Concerning changes at time-point 12 h after challenge, there is no comparable data in the literature. The occurrence of different patterns of late serum peaks may be due to inter-individual variation and probably does not indicate different pathomechanisms.

One patient (Fig 1, No. 12) showed a marked increase of both mediators despite a lack of any clinical reaction. One argument for the early serum increase of ECP/EPX in this patient is that response to the allergen challenge might have been "sub-clinical" (FEV₁ < 20%). In this case, monitoring of allergen challenge by mediator measurement might have been more sensitive than lung function (33).

In contrast, another patient (Fig. 5, No. 26), who represents the small group 5, showed no variation in ECP/EPX serum concentration despite a significant dual asthmatic reaction (DAR). This phenomenon is difficult to interpret: one might hypothesize that in these cases eosinophil activation is less important for the development of an EAR or LAR.

ECP and EPX serum concentration showed a striking parallel time-course. Both mediators seem to use the same releasing mechanism, as suspected from *in vitro* experiments (34). Measuring both mediators does not seem to be necessary for clinical purposes.

From our results we conclude that the striking groups of time-courses of ECP/EPX serum concentration indicate different uniform patterns of eosinophil activation during allergen challenge – but do not predict clinical outcome of provocation. Further studies (especially in BAL) are needed to establish the role of the eosinophil in the early asthmatic reaction.

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