

## CLINICAL REPORT

## Longitudinal Case Analysis in Atopic Dermatitis

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The current knowledge on atopic dermatitis comes mainly from cross-sectional studies, which are not suited to establish time-courses or causal links in complex diseases. As an alternative approach, the method of longitudinal case analysis by the autoregressive integrated moving average (ARIMA) method has been introduced to investigate the pathogenesis of atopic dermatitis. The method was applied to the investigation of 2 patients suffering from severe and moderate atopic dermatitis. Disease activity, peripheral blood parameters (differential blood count, lymphocyte subpopulations, immunoglobulin E, eosinophilic cationic protein, soluble interleukin-2 receptor), mental stress and environmental factors were determined daily for 50 days. Both patients showed a positive correlation between CD4<sup>+</sup> and CD25<sup>+</sup> T-cells, a negative correlation between CD16/56<sup>+</sup> natural killer cells and CD4<sup>+</sup> T-cells, a negative correlation between eosinophils and polymorphonuclear leukocytes, and a time-shifted positive correlation of up to 2 days between scores quantifying mental stress and disease activity. A positive correlation between T-cells and polymorphonuclear leukocytes, CD4<sup>+</sup> T-cells and the CD45RA<sup>+</sup> subtype, as well as a negative correlation between stress and eosinophils, sports and eosinophils, and sports and disease activity were found only in one patient with more severe atopic dermatitis. In conclusion, longitudinal time-series analysis might represent an interesting and adequate method to generate and test new hypotheses on biomedical problems which cannot be addressed by cross-sectional studies. **Key words:** *time-series analysis; ARIMA; atopic eczema; neurodermitis.*

(Accepted August 14, 2000.)

Acta Derm Venereol 2000; 80: 348–352.

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The present knowledge on the pathophysiology of atopic dermatitis (AD) has to take various parameters into account (1). Besides the imbalance of lymphocyte subpopulations and the presence of specific immunoglobulin E (IgE), nearly all cells related to the immune system and the skin (granulocytes, monocytes, keratinocytes, Langerhans' cells, mast cells), cytokines and receptors as well as adhesion molecules are involved in this scenario. The individual course of the disease is triggered by different factors (2) which can be divided into 4 main pathogenic variables: allergens (exogenous contact, nutritive or inhalant allergens), non-specific influences (i.e. all types of skin irritation), local infections (e.g. *Staphylococcus aureus*) and mental factors (e.g. stress, lifestyle). Although there have been promising attempts, a general pathogenetic concept connecting the various findings is still lacking (3).

The most frequently applied methods for the investigation of the pathogenesis of AD have been cross-sectional studies. A typical design of a cross-sectional study might be, for instance, to analyse a disease factor in 50–100 patients with AD compared with an adequate control population. Cross-sectional investigations are excellent tools to search for potential new disease parameters and to gain more detailed knowledge. Nevertheless, they usually fail to explain the relations and relevance of the findings. The idea behind the present investigation was to focus on 2 patients' comparable effort and expenses to those used in a 100-patient cross-sectional study, to obtain detailed, cinematograph-like time-course information on the interrelated orchestration of important disease-specific parameters. Subsequently, the method of longitudinal time-series autoregressive integrated moving average (ARIMA) analysis was introduced to the ongoing cell biology research on AD.

This study addresses the question of whether the ARIMA method is suitable for gaining new insights into the pathogenesis of AD and providing new findings on the fine-tuning of this method (i.e. selection of parameters and best measurement frequency) applied in AD research.

## MATERIAL AND METHODS

*Study design*

Two patients suffering from AD answered 5 questionnaires scoring disease activity, mental stress, allergen contact, skin irritation and lifestyle daily for a period of 50 days. Daily, blood was drawn from the forearm (8.00–9.00 a.m., before the first meal) for laboratory investigations (see below). The patients were living in their usual environment and following regular activities. The patients were advised to avoid contact with known allergens for the whole study period. Only innocuous, urea or tar-containing ointments were allowed for skin care.

*Patients*

Patient 1 was a 21-year-old female student who has suffered from AD since her first year of life. She also suffered from mild seasonal allergic rhinoconjunctivitis. There was no family history of AD. For the past 2 years, the patient has been suffering continuously from severe skin involvement. At the beginning of the study clinical examination revealed extensive eczema with 54% involvement of the total body surface affecting both hands, the flexural and lateral skin of the extremities, the whole face and neck, and the upper trunk. Physical examination, case history and general medical laboratory values (liver enzymes, renal laboratory tests, urinary sediment, erythrocyte sedimentation rate, complete blood count, blood glucose, serum protein and coagulation laboratory tests) revealed no reference to any other disease. The patient was a non-smoker, not a drug addict and does not use any medical drugs or contraceptives. Specific IgE tests (CAP-RAST, Pharmacia & Upjohn, Sweden) revealed *Dermatophy-*

*goides pteronyssinus* class III, *Dermatophagoide s farinae* class II, birch pollen class III, and beech, poplar, elm tree and rye pollen class II. Prick tests showed positive reactions to the first 3 and also to ragweed.

Patient 2 was a 23-year-old female student who has suffered from AD since her 12th year of life. No additional atopic diseases were reported. For the past 2 years, the patient has been suffering from 3 extended episodes of AD (up to 3 months' duration). At the beginning of the study the patient presented with an itching bilateral eczema of the flexural skin of the elbows, knees and anterior neck (12% of the total body surface). Physical examination, case history and general medical laboratory values did not show any other disease. The patient was non-smoker, not a drug addict and was not taking any medical drugs or contraceptives. A prick test with inhalant allergens showed positive reactions to *D. pteronyssinus* and cat dander. Specific IgE tests revealed *D. pteronyssinus* class III.

In both patients, the diagnosis of AD was based on the features given by Hanifin & Rajka (4). Clinical examination was complemented by psychometric tests: the Giessen Test (5), Marburg Skin Disease Coping Questionnaire (6), Leipzig Questionnaire for Social Risks (7) and Questionnaire for Physical Complaints (8). The personality features of both patients were in the normal range. Both patients were psychologically healthy, had a normal coping style, and had no remarkable social or psychosomatic risks except for a relatively high professional stress level and type A behaviour in patient 1.

*Laboratory methods*

*Complete blood count.* Heparinized venous blood 1.5 ml was used daily for a standardized automatic analysis (Coulter STKS) of absolute leukocyte numbers and the relative amounts of polymorphonuclear leukocytes (PMN), eosinophilic and basophilic granulocytes, lymphocytes and monocytes.

*Phenotyping of lymphocytes by the fluorescence activated cell sorter (FACS).* Heparinized venous blood 100 µl was required per antibody pair or triplet and analysed daily (chemicals, antibodies, FACS equipment and software: Becton Dickinson, Heidelberg, Germany). The following antibody combinations were used: CD3-CD19, CD3-CD4, CD3-CD8, CD3-CD16/56, CD3-HLA-DR, CD25-CD3, CD19-CD23, CD45RA-CD45RO-CD4. Antibodies were labelled with fluorescein isothiocyanate (FITC)-phycoerythrin (PE); in the last case, anti-CD4 was labelled with peridinin chlorophyll protein (PerCP) by the triple fluorescence technique.

*Total IgE levels, eosinophilic cationic protein (ECP) and soluble interleukin-2 receptor (sIL-2R).* Serum samples and collection methods have been described in detail previously (9). ECP and sIL-2R were determined in patient 2 only.

*Daily questionnaires*

*Disease activity.* The SCORAD index of the European Task Force on Atopic Dermatitis (10) was used as a self-managed instrument documenting disease activity. After instruction and a supervised exercise period it was completed by the patients each day.

*Mental stress.* Sixty items reflecting daily stress were recorded using the Daily Stress Inventory (DSI), German version (11).

*Allergen contact and skin irritation.* A self-developed and previously evaluated daily questionnaire was used to register allergen exposure of potential clinical relevance as well as contact to skin irritants, disease-promoting activities or climate influences.

*Lifestyle.* A self-developed questionnaire was used with scales for daily quantification of working hours and working intensity, dura-

tion of sleep, sleep quality, sports, alcohol, nicotine, sexual behaviour and menses.

*Statistics*

For each of the total of 34 parameters a time-series of 50 values (1 value per day) was constituted. Time-series were investigated for the direct or bidirectional 1, 2, 3, 4 and 5 days' time-shifted cross-correlation to detect delayed correlations. For this purpose, possibly independent parameters were defined as "lead" and dependent as "lag" variables according to the following hypotheses. (i) Each laboratory parameter (see above) could influence each other laboratory parameter and disease activity. (ii) Mental stress and lifestyle parameters could influence each laboratory parameter and disease activity. (iii) Disease activity, mental stress and lifestyle parameters could influence each other. (iv) Allergen contact and exogenous skin irritation could influence all other parameters but are not affected by them.

Because serial and tendency dependencies of time-series often cause too high correlation coefficients when applying "simple" cross-correlation analysis, a special statistical filter procedure, the ARIMA method, was used to release the time-series from perturbing influences and pseudodependencies, e.g. weekly rhythms (12, 13). Appropriate ARIMA models were identified for all time-series of time-series pairs showing significant pre-ARIMA cross-correlation. Time-series were then filtered by the ARIMA model of the corresponding lead variables (Fig. 1), after which the residues of lead and lag variables underwent a time-shifted correlation analysis. BMDP software was used in the analyses (Statistical Software, Los Angeles, CA, USA).

RESULTS

Out of the total of 62 time-series in the study (30 in patient 1 and 32 in patient 2), 18 series were involved in significant correlations with other time-series (10 vs 8). Characteristically pairs of correlative time-series are given in Fig. 2. Most of the measured parameters were applicable for statistical analysis by the ARIMA method. Exceptions were the IgE level

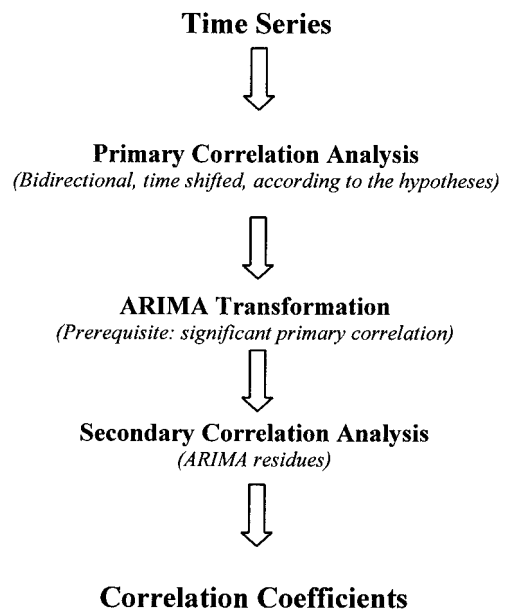


Fig. 1. Flow chart of the statistical analysis method applied in this study. For efficient analysis of many parameters, a primary deselection of non-correlating matches is necessary. ARIMA: autoregressive integrated moving average.

(significant changes were detectable within weeks only), as well as sIL-2R and ECP levels (no day-to-day trend stability).

To determine the usefulness of the applied method, it is necessary to look for the single outcome variables first. Then, the intervariable relations are given as correlation coefficients associated with their level of statistical random error (see Table I and the *Correlation data* section).

#### Patient 1

During the investigation, the involved eczematous skin area (scale A of the SCORAD index) ranged from 43 to 65% (mean  $\pm$  SD:  $55.1 \pm 5.5$ ) of the body surface, corresponding to high total SCORAD index values between 41.1 and 79.9 ( $62.08 \pm 9.02$ ) with 2 maxima at day 5 and between day 19 and 24, and a minimum at day 39. This state was reflected by laboratory parameters such as eosinophilia ( $18.39 \pm 4.04\%$ ), and very high and slowly changing IgE levels ( $4577 \pm 146.4$  kU/l). Concerning the lymphocyte subpopulations, a slight elevation of CD3<sup>+</sup> CD4<sup>+</sup> helper T-cells (Th) ( $55.1 \pm 6.52\%$ ) was accompanied by a marked decrease in CD3<sup>+</sup> CD8<sup>+</sup> suppressor/cytotoxic T-cells ( $16.1 \pm 1.32\%$ ). The majority of T-cells exhibited the phenotype of memory cells (CD45R0<sup>+</sup>):  $46.3 \pm 5.35\%$ . The T-cell activation marker CD25<sup>+</sup> and HLA-DR were remarkably expressed:  $14.95 \pm 1.76\%$  and  $13.55 \pm 1.94\%$ , respectively. Approximately half of the CD19<sup>+</sup> B-cells ( $12.63 \pm 2.14\%$ ) expressed the low-affinity IgE receptor CD23:  $6.50 \pm 2.31\%$ . Scores for mental stress (DSI) showed high values: 29–112 ( $67.0 \pm 16.66$ ).

#### Patient 2

During the investigation, the skin area involved ranged from 12 to 18% of the total body surface ( $15.2 \pm 1.47$ ), and the total SCORAD Index showed values between 18.8 and 70.4

( $45.94 \pm 14.38$ ) with 5 distinct maxima distributed evenly over the duration of the study. Concordant with the minor severity of the disease in patient 2, few specific findings were detectable in the peripheral blood. As in patient 1, CD8<sup>+</sup> suppressor/cytotoxic T-cells were decreased:  $17.6 \pm 1.51\%$ . In contrast to patient 1, the relative number of CD19<sup>+</sup> B-cells ( $17.32 \pm 2.24\%$ ) as well as CD16/56<sup>+</sup> natural killer (NK) cells ( $16.5 \pm 3.81\%$ ) were increased. The values of ECP and sIL2-R were also increased:  $28.4 \pm 14.1$   $\mu$ g/l, and  $574.02 \pm 288.33$   $\mu$ g/l, respectively. The scores of mental stress (DSI) were in a similar range to patient 1: 18–122 (mean  $78.1 \pm 29.34$ ).

#### Correlation data

As shown in Table I, a significant positive correlation between Th and CD25<sup>+</sup> activated T-cells could be demonstrated in both patients. This was accompanied by a very strong negative association of T-cells or Th and CD16/56<sup>+</sup> natural killer (NK) cells and a negative association of eosinophils and PMN values.

With respect to the non-laboratory parameters, in both patients, a significant positive correlation between mental stress and disease activity could be demonstrated. However, direct correlation of these values appeared only in patient 2, while it occurred with a time-shift of 2 days in patient 1.

In patient 1 (with a higher disease activity) a larger number of correlative associations could be demonstrated than in patient 2. Only in patient 1, a significant negative association was found between the scores of stress or sports and counts of blood eosinophils. Equivalently to the above-mentioned time-shift in the association between mental stress and disease activity, a 2 days' time-shifted significant negative association between eosinophils and disease activity was evident. Furthermore, patient 1 showed a direct positive correlation between Th and its CD45RA<sup>+</sup> (naive) subpopulation. A complete list of significant correlation coefficients is given in Table I. No influence of

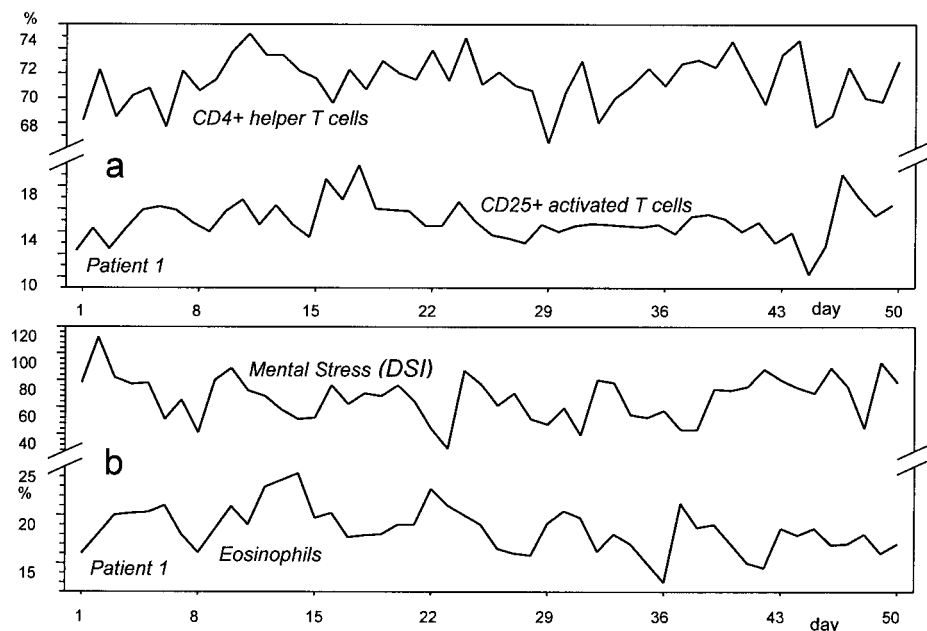


Fig. 2. Examples of highly significant correlating time-series in patient 1. (a) Positive correlation of the counts of helper T-cells with CD25<sup>+</sup> activated T-cells (correlation coefficient + 0.499,  $p \leq 0.001$ ). (b) Negative correlation of mental stress (Daily Stress Inventory, dimensionless) and counts of eosinophils (correlation coefficient - 0.422;  $p \leq 0.01$ ).

allergen contact, exogenous skin irritation or lifestyle on laboratory parameters or disease severity could be determined.

DISCUSSION

The time-series analysis by the ARIMA method was developed in the 1970s for technical and economical process analysis (i.e. passenger forecasting in aircrafts). It has been successfully applied in psychological sciences, psychodermatology and general epidemiology (14–17). Today, it represents the method of choice in the treatment of time-series and is considered superior to other statistical methods. The application of time-series analysis in medical sciences provides a unique possibility: statistical accuracy in the analysis of the time-course of a disease (in single cases). However, the results are primarily significant only in the investigated patients. Thus, this study represents 2 extended and extraordinarily detailed case reports. Some important results to determine the quality of the method will be discussed.

In both patients, the peripheral blood CD4<sup>+</sup> Th population was upregulated. This is a common observation in patients suffering from AD (18). Interestingly, the present data show a strong association between Th counts and the expression of the activation marker CD25 on blood T-cells, while another important molecule expressed on activated T-cells, HLA-DR, showed no strong association to the Th counts in the

investigated patients. The activation marker CD25 represents the interleukin-2 (IL-2) receptor  $\alpha$ -chain. Therefore, a co-ordinated upregulation of CD25<sup>+</sup> T-cells and the whole Th population might explain this observation. Although HLA-DR<sup>+</sup> expression was found to be more frequently increased than other activation markers in AD patients (19), these results suggest that CD25 may play a more important functional role than HLA-DR in the present patients. Furthermore, the lack of association between CD25 expression and menstruation in these 2 female patients does not support an influence of gonadal axis hormones on CD25 expression (20).

In these patients, the peripheral CD4<sup>+</sup> Th population (and total CD3<sup>+</sup> T-cells) were negatively correlated with CD16/56<sup>+</sup> NK cell counts. These results correspond to the observation that NK cell activity and concentration are commonly reduced in the peripheral blood of patients suffering from AD (21). In addition, they support published observations linking NK cell development (via IL-12 and interferon- $\alpha$  and - $\psi$  production) to the Th1 cytokine pattern rather than to the Th2 pattern of AD (22).

Mental stress had a significant impact on disease activity in these 2 patients. This result confirms other findings which showed the worsening of AD following mental stress by time-series analyses (14) and data from cross-sectional studies focusing on mental stress and AD (23, 24).

Table I. Significant positive and negative correlation coefficients of disease parameters in patients 1 and 2

Lead <sup>a</sup>	Lag <sup>b</sup>	Patient	Lag 0	Lag 1	Lag 2
CD4 <sup>+</sup> T-cells	CD25 <sup>+</sup> T-cells	1	+ 0.499***	– <sup>c</sup>	–
		2	+ 0.305*	+ 0.288*	–
CD25 <sup>+</sup> T-cells	CD4 <sup>+</sup> T-cells	1	+ 0.499***	–	–
		2	+ 0.305*	+ 0.327*	–
CD4 <sup>+</sup> T-cells	CD16/56 NK cells	1	– 0.509***	–	–
		2	– 0.758***	–	–
CD16/56 NK cells	CD4 <sup>+</sup> T-cells	1	– 0.509***	–	–
		2	– 0.758***	–	–
Total T-cells	CD16/56 NK cells	1	– 0.483***	–	–
		2	– 0.574***	–	–
CD16/56 NK cells	Total T-cells	1	– 0.483***	–	–
		2	– 0.574***	–	–
CD4 <sup>+</sup> T-cells	CD4 <sup>+</sup> CD45RA <sup>+</sup> T-cells	1	+ 0.316*	+ 0.382**	–
		2	–	–	–
CD4 <sup>+</sup> CD45RA <sup>+</sup> T-cells	CD4 <sup>+</sup> T-cells	1	+ 0.316*	–	–
		2	–	–	–
Eosinophils	Polymorphonuclear granulocytes	1	– 0.516***	–	–
		2	– 0.344*	–	–
Polymorphonuclear granulocytes	Eosinophils	1	– 0.516***	–	–
		2	– 0.344*	–	–
Mental stress	Eosinophils	1	– 0.422**	–	–
		2	–	–	–
Mental stress	SCORAD index	1	–	–	+ 0.282*
		2	+ 0.491***	–	–
Eosinophils	SCORAD index	1	–	–	– 0.350*
		2	–	–	–
Sports	Eosinophils	1	– 0.508***	–	–
		2	–	–	–

<sup>a</sup>Lead: independent “leading” parameter according to the hypotheses.

<sup>b</sup>Lag: dependent parameter.

Lag 0, 1, 2: time-shifting correlation analysis: lag 0, no time-shift; lag 1, 1 day’s; lag 2, 2 days’ time-shift.

<sup>c</sup>not significant.

\* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ . NK: natural killer

In patient 1 with more severe AD, the concentration of eosinophils was negatively associated with mental stress. Moreover, counts of eosinophils were negatively correlated with disease activity in a 2-day time-shifted manner. These observations were paralleled by the influence of mental stress on disease activity in this patient. The influence of mental stress on the counts of eosinophils and activity is poorly understood. Some almost forgotten papers from the 1970s have reported a stress-inducible eosinopenia (25, 26). The present data, together with knowledge on the early involvement of eosinophils in AD skin lesions (27) might support the hypothesis that an infiltration of the skin and other tissues, paralleled by decreasing counts of eosinophils in the blood through mental stress may reflect a link between psychological and immunoregulatory events (28).

For statistical reasons, a minimum of 50 measurements is necessary in each time-series to apply the ARIMA method. Determining the optimal frequency collecting the data is one of the most important problems in longitudinal case analysis. The half-life of the different peripheral blood parameters was taken into account for the design of the study. Together with the questionnaires used, a daily measurement was predicted as the optimum for most of the parameters. As expected, IgE levels as well as sIL-2R or ECP seemed to require less or more frequent measurements, respectively.

In summary, the results of this study are coherent with the present knowledge of AD. In addition, some detailed findings in these 2 patients permit the generation of new hypotheses. Thus, the method of time-series analysis seems to be a promising tool to study the relationships of various parameters in complex diseases and to strengthen the recognition of variables which might be functionally important, in contrast to irrelevant simple epiphenomena. However, enthusiasm is limited by the extreme expenses of this method. The investigation of 2 single cases by time-series analysis demonstrates a novel and interesting approach to investigate the pathophysiology of a chronically relapsing and complex skin disease such as AD.

#### ACKNOWLEDGEMENT

We thank Dr Jörg Kleine-Tebbe, MD (Department of Dermatology, University of Leipzig, Germany) for editorial assistance and insightful comments.

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