

Report

High serum APRIL levels strongly correlate with disease severity in pediatric atopic eczema

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Abstract

Background Expression of the tumor necrosis factor (TNF) family member APRIL (a proliferation-inducing ligand) is low in normal tissues but is elevated in various autoimmune diseases.

Objectives This study explored serum APRIL in children with atopic eczema (AE) during flare and quiescence.

Methods A case-control study including 50 patients with AE and 40 control subjects was conducted. The severity of AE was assessed according to each of the Leicester Sign Score (LSS), the Simple Scoring System (SSS), the SCORing of Atopic Dermatitis (SCORAD) index, and the Objective SCORAD. Serum measurements of APRIL, total immunoglobulin E, and lactate dehydrogenase were obtained in all subjects. Data were obtained during both flare and quiescence in AE subjects. Data were analyzed using the Mann-Whitney *U*-test and Pearson's correlation coefficient. *P*-values of <0.05 were considered to indicate statistical significance.

Results Serum APRIL levels were significantly elevated in children with AE in comparison with control subjects during both flare and quiescence (*P* < 0.0001 for both). Serum APRIL levels during AE flare and quiescence were positively correlated (*P* < 0.001). Serum APRIL levels and scores on each of the LSS, SSS, and SCORAD showed significant positive correlations (*P* < 0.01 for all).

Conclusions Serum APRIL levels were significantly elevated in children with AE during both flare and quiescence. This confirms the importance of APRIL in the pathophysiology of pediatric AE. Serum APRIL is a reliable marker of the severity of AE in children. APRIL may be a new target in the treatment of AE.

Introduction

The tumor necrosis factor (TNF) family is intimately connected to the regulation of cellular pathways. The TNF ligand members BAFF (B cell activating factor) and APRIL (a proliferation-inducing ligand) are two factors crucial to the survival of peripheral B cells and are also termed B lymphocyte stimulators (BLyS). Both ligands can bind two members of the TNF receptor family, namely, transmembrane activator and calcium modulator cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA). In addition, APRIL mostly exerts its effects through binding to HSPG (heparan sulfate proteoglycans) and can promote antibody class switching independently of the CD40/CD40L pathway.¹

APRIL, a member of the TNF ligand superfamily (TNFSF13), is a type II transmembrane protein. It is named for its capacity to stimulate the proliferation of

tumor cells *in vitro*. APRIL is expressed mainly by innate immune cells and to a lesser extent by T cells and activated B cells, as well as by non-hematopoietic tissue resident cells. APRIL expression is low in normal tissues but is elevated in several types of tumor and transformed cell lines.²

APRIL functions only as a soluble protein. Immune histological analyses have confirmed an intracellular expression pattern of APRIL in keratinocytes, both *in vivo* and *ex vivo*, which differs from the cell membrane-associated expression pattern of BAFF. BAFF is expressed as both surface-bound and soluble factors, whereas APRIL is processed inside the cell and released as a soluble protein. However, APRIL can be attached to the cell surface by a process of natural fusion with the protein TWEAK (TNF-like weak inducer of apoptosis), which shares receptors with APRIL.³ This process is called TWE-PRIL.

In humans, increased levels of APRIL in serum or target tissues have been described in lymphoid cancers^{4–7} and in a number of autoimmune diseases, particularly systemic lupus erythematosus^{8–12} and rheumatoid arthritis,^{13–15} and are often correlated to disease progression and severity. In atopic diseases, elevated serum levels of BAFF have been reported as diagnostic parameters for asthma¹⁶ and pediatric atopic eczema (AE).¹⁷ Other researchers have published data revealing elevations in serum levels of APRIL, but not of BAFF, in adult AE.^{18,19}

Notably, no data on the expression of APRIL in biological fluids and/or target tissues in pediatric AE have been reported. Therefore, we sought to explore the expression of APRIL in the sera of children with AE during flare and quiescence and to identify the clinical relevance of its measurement in terms of disease activity and severity.

Materials and methods

This follow-up case-controlled study comprised 90 children, including 50 patients with AE diagnosed by standardized diagnostic criteria,²⁰ and 40 clinically healthy children recruited to serve as controls. The study was approved by the ethics committee of Ain Shams University and conformed to standards currently applied in Egypt. All patients were enrolled at the Pediatric Allergy and Immunology Unit and Outpatient Clinic of Ain Shams University Children's Hospital, Cairo, Egypt. An informed written consent document was obtained from the parents of each child before enrollment according to the Declaration of Helsinki.

Criteria for exclusion of children with AE

Patients with AE were enrolled only after any past, current, or family history of other atopic diseases, such as asthma, allergic rhinitis, and allergic conjunctivitis, had been excluded.

Patients with any other dermatological illnesses were excluded.

Patients receiving systemic corticosteroids or immunosuppressive therapy were excluded (under a definitive exclusion criterion). Topical corticosteroids and antihistamines were stopped for at least 2 weeks before entry to the study. Only topical emollients were used.

Criteria for inclusion of healthy controls

Healthy control subjects were enrolled after a detailed history had been taken and a thorough clinical examination conducted to exclude any systemic or dermatological illnesses, with special emphasis on hematological malignancies, rheumatic disorders, and atopic diseases such as AE, asthma, allergic rhinitis and allergic conjunctivitis.

Subjects with peripheral blood eosinophilia or elevated serum immunoglobulin E (IgE) for age were excluded.

Assessment of the clinical severity of AE

For convenience, the severity of AE was assessed according to four different scoring systems.

The Leicester Sign Score (LSS) calculates severity according to six clinical features (erythema, purulence, excoriation/crusting, dryness/scaling, cracking/fissuring, and lichenification) graded at each of six defined body sites on a scale of 0 (none) to 3 (severe) (LSS score range: 0–108).²¹

The Simple Scoring System (SSS) described by Costa *et al.*²² calculates the intensity of 10 signs and symptoms along with involvement of 10 different skin regions. The 10 indications refer to erythema, edema, vesicles, crusts, excoriation, scales, lichenification, pigmentation or depigmentation, pruritus, and loss of sleep. The maximum score possible is 100 points.²²

The SCORing of Atopic Dermatitis (SCORAD) index was used to evaluate the severity of eczema by combining two objective symptoms (extent and intensity of eczema) and two subjective symptoms (pruritus and sleep loss) to a maximum score of 103 points.²³

The Objective SCORAD is a modification of the SCORAD that excludes subjective symptoms such as pruritus and sleep loss in order to minimize errors caused by variability in patients' ages and backgrounds. A proposal for the grading of severity of AE using only objective criteria is as follows: mild AE: scores of <15; moderate AE: scores of 15–40; severe AE: scores of >40.²⁴

Study design

At enrollment, all children with AE were clinically evaluated for the severity of AE while presenting with eczematous flare and then sampled for APRIL assay. Patients were followed up until the quiescence of AE had been achieved through the use of topical emollients only (topical steroids and oral antihistamines were stopped for at least 2 weeks before sampling), at which point follow-up blood samples were collected. Quiescence and the response to treatment were scored using the Physician's Global Assessment (PGA) scale. The PGA is an overall assessment made on a scale of 0–5 of a patient's eczema, which takes into consideration the quality and extent of lesions relative to the baseline assessment [0 = clear (100%); 1 = almost clear (90–99% improvement); 2 = marked improvement (50–89%); 3 = modest improvement (<50%); 4 = no change; and 5 = worse]. Quiescence was achieved after a mean $\pm$ standard deviation (SD) duration of 4.0 ± 1.2 weeks. It is noteworthy that the rate of patient dropout was nil.

Study measurements

Quantitative determination of serum human APRIL (TNFSF13)

Reagents (Quantikine®, catalog no. DY884) were supplied by R&D Systems, Inc. (Minneapolis, MN, USA).

Serum total IgE

Serum total IgE was assayed in all subjects by quantitative enzyme immunoassay. Results were expressed in IU/ml. In view of the variability in serum total IgE levels with age in childhood, percentage values were calculated from the reference ranges by dividing the subject's actual level by the highest normal level for age multiplied by 100.²⁵ IgE levels used in the correlations were both the measured and the calculated percentage values. Any serum total IgE level that exceeded the highest normal level for age was considered elevated.

Serum total lactate dehydrogenase assay

Serum total lactate dehydrogenase (LDH) (U/l) was measured in the AE group during flare and in the control subjects.

Complete blood count

A complete blood count was performed in all subjects using the Coulter counter. Differential leukocyte counts were estimated manually from blood film and expressed in absolute count values. Children in whom absolute eosinophil counts (AECs) exceeded normal reference values for age were considered to have peripheral blood eosinophilia.²⁵ Blood sampling of subjects was performed at the same time (10.00 hours) each day to avoid diurnal variations in eosinophil counts.

Stool and urine analyses

Stool and urine analyses were performed to exclude parasitic infestations when suspected.

Statistical analyses

All statistical analyses of the data were carried out using SPSS for Windows Version 11.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as the mean $\pm$ SD, median, and interquartile range (IQR; 25th and 75th percentiles). For non-parametric comparisons of serum APRIL among different groups, the Mann–Whitney *U*-test was used. The relationships among the various numerical parameters were studied using the Pearson correlation coefficient (*r*) test with graphic representation using the linear regression line; an *r*-value was considered weak at $r < 0.50$, moderate if *r* ranged between 0.50 and 0.75, and strong at $r > 0.75$. *P*-values of <0.05 were considered to indicate differences of statistical significance in all tests.

Results

Demographic, clinical, and laboratory data for AE patients during flare and for controls are shown in Table 1.

Serum APRIL expression was not influenced by age, gender, or family history. Serum APRIL levels could not be correlated with age in either the AE or the control group ($P > 0.05$). Serum APRIL levels were statistically comparable among males and females in both the AE and

Table 1 Demographic, clinical, and laboratory data for atopic eczema (AE) patients during flare and control subjects

Variable	AE patients (<i>n</i> = 50)	Controls (<i>n</i> = 40)
Age ^a , months	36.12 $\pm$ 12.63 (12–96)	33.73 $\pm$ 13.02 (16–84)
Male/female, <i>n</i>	26/24	19/21
Duration of illness ^a , months	16.87 $\pm$ 9.63 (4–48)	–
Severity, Leicester Sign Score ^a	56.24 $\pm$ 17.83 (17–92)	–
Severity, Simple Scoring System ^a	50.60 $\pm$ 20.30 (10–90)	–
Severity, SCORAD index ^a	30.65 $\pm$ 10.27 (10–60)	–
Severity, Objective SCORAD ^a	26.74 $\pm$ 8.98 (9–45)	–
Absolute eosinophil count ^a , 10 ⁹ /L	523.82 $\pm$ 125.87 (378–945)	85.34 $\pm$ 15.77 ^b (55–120)
Serum total IgE ^a , IU/ml	200.00 $\pm$ 60.67 (25–405)	21.00 $\pm$ 8.54 ^b (9–60)
Serum total LDH ^a , U/l	1010.87 $\pm$ 325.54 (157–1922)	90.76 $\pm$ 22.87 ^b (35–199)

^aMean $\pm$ standard deviation (range).

^bStatistically significant difference ($P < 0.05$).

IgE, immunoglobulin E; LDH, lactate dehydrogenase; SCORAD, SCORing of Atopic Dermatitis.

control groups ($P > 0.05$). When AE patients and controls were compared with respect to serum total LDH, serum total IgE, and AEC, statistically significant differences were detected ($P < 0.05$ for all) (Table 1).

Serum APRIL levels were significantly elevated in children with AE in comparison with control subjects during both flare and quiescence ($P < 0.0001$ for both) (Fig. 1).

A positive correlation was elicited between serum APRIL levels during AE flare and the corresponding values during quiescence ($r = 0.65$, $P < 0.001$) (Fig. 2).

According to the SCORAD index of severity, 20 (40%) of the study patients had moderate AE, and the remaining 30 (60%) had severe AE. On the Objective SCORAD index, 10 (20%) had mild AE, 12 (24%) had moderate AE, and 28 (56%) had severe AE.

According to the Objective SCORAD index of severity, the median and mean $\pm$ SD levels of serum APRIL in the severe AE group (3050 pg/ml, 2689 $\pm$ 1543 pg/ml) and the moderate AE group (2640 pg/ml, 1676 $\pm$ 969 pg/ml) were significantly higher than the corresponding values in the mild AE group (850 pg/ml, 620 $\pm$ 320 pg/ml) (Fig. 3).

There were significant positive correlations between serum APRIL levels and scores on each of the LSS (Fig. 4), SSS (Fig. 5), and SCORAD (Fig. 6) indices of severity. It is noteworthy that the correlation between

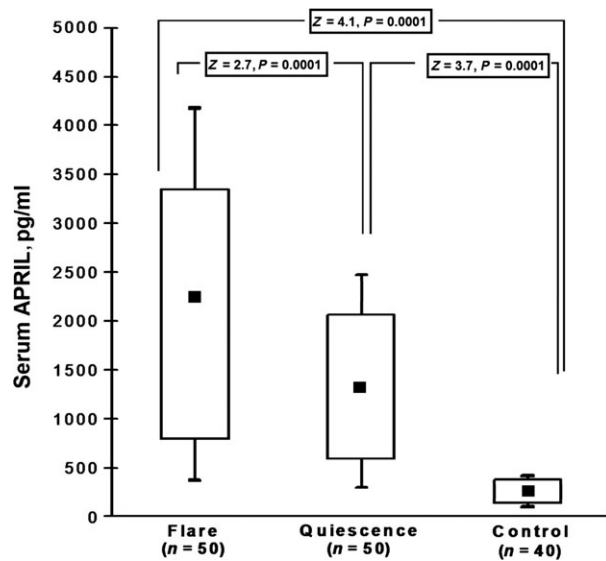


Figure 1 Summary of serum APRIL levels in flare and quiescence in pediatric subjects with atopic eczema ($n = 50$), and in control subjects ($n = 40$). The Mann–Whitney test was used in the comparisons. Black squares represent medians; boxes encompass the interquartile range (25th and 75th percentiles). Ranges are marked as the maximum and minimum

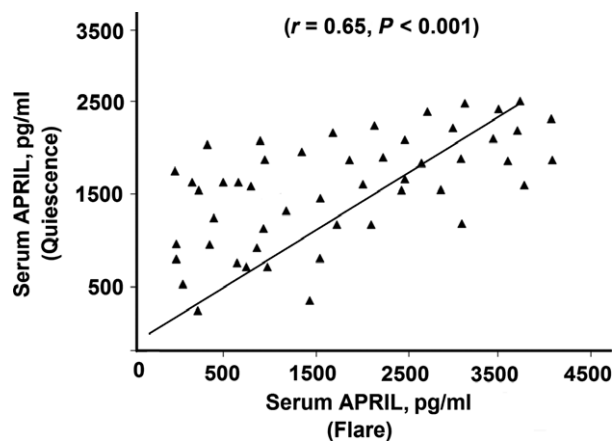


Figure 2 Serum APRIL levels during flare and quiescence in pediatric patients with atopic eczema ($n = 50$) show a positive correlation

serum APRIL and the Objective SCORAD index was insignificant.

There were no significant correlations between serum APRIL levels and serum total IgE level or AEC in the AE group. Serum total LDH levels correlated positively with serum APRIL levels ($r = 0.62$, $P < 0.01$) and with the LSS ($r = 0.59$, $P < 0.05$) and SCORAD ($r = 0.52$, $P < 0.05$) indices of severity.

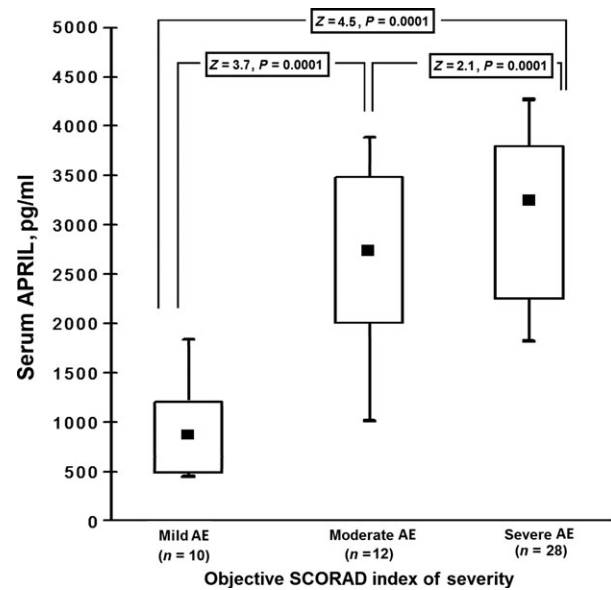


Figure 3 Summary of serum APRIL levels in pediatric atopic eczema (AE) patients ($n = 50$) categorized by severity according to the Objective SCORAD index of severity

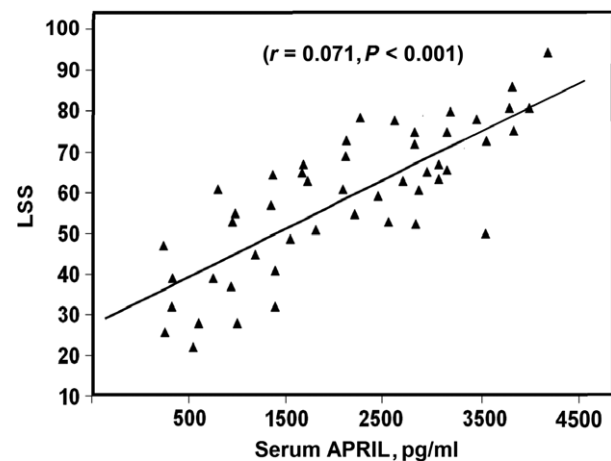


Figure 4 Serum APRIL levels and Leicester Sign Score (LSS) indices of atopic eczema (AE) severity in pediatric AE patients ($n = 50$) show a significant positive correlation

Discussion

Published data in adults with AE revealed elevated APRIL serum levels.^{18,19} Immunohistological analysis confirmed an intracellular expression pattern of APRIL in adult keratinocytes both *in vivo* and *ex vivo*, in comparison with a cell membrane-associated expression pattern of other TNF ligands.^{26,27} However, information on APRIL expression in the biological fluids and/or target tissues of children with AE is limited.

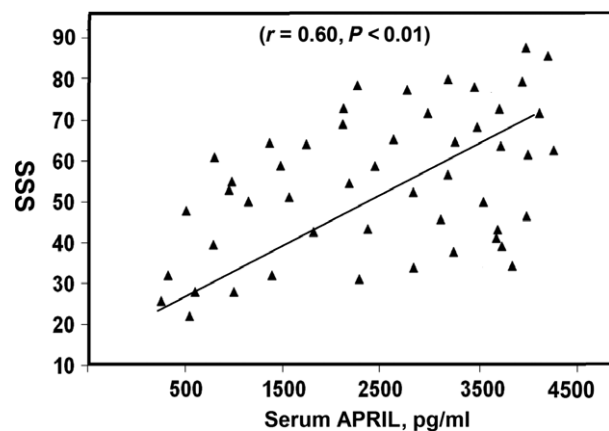


Figure 5 Serum APRIL levels and Simple Scoring System (SSS) indices of atopic eczema (AE) severity in pediatric AE patients ($n = 50$) show a significant positive correlation

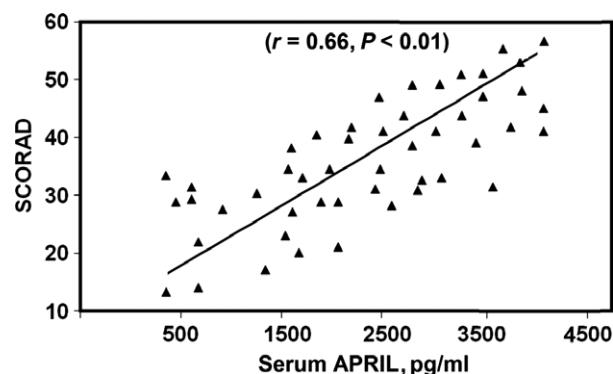


Figure 6 Serum APRIL levels and SCORAD (SCORing of Atopic Dermatitis) indices of atopic eczema (AE) severity in pediatric AE patients ($n = 50$) show a significant positive correlation

The novelty of the present study refers to its use of a pediatric patient group. Here, we sought to define the role of APRIL in AE by comparing serum levels during flare and quiescence, as well with those in healthy controls, and exploring the relevance of serum levels to disease activity and severity indices.

We observed that serum APRIL levels were significantly elevated in children with AE during both flare and quiescence in comparison with those in controls. Our results confirm the importance of APRIL in the pathophysiology of pediatric AE. The role of B cells in the pathology of AE is indisputable, and their contribution beyond IgE production must be appreciated. Factors influencing B cell activation and homeostasis in AE, in both systemic and target tissue environments, require to be characterized. The APRIL signaling pathway has an important role in the selection, development, proliferation, maturation, and

survival of B cells, subsequently leading to antibody class switching. APRIL may also play an important role in T cell activation and survival.²⁸ APRIL overexpression may result in dysregulation or over-activated B cells and/or the reduction of a negative regulatory input to B cells, and possibly to the development of AE. This was found to be more pronounced in the initial acute stage of AE and represents a potentially predictive useful marker for the presence of an atopic response.

APRIL can be expressed by resident cells in several kinds of tissue. Healthy keratinocytes can synthesize and express APRIL, both *in vivo* and *ex vivo*, in lesional skin of AE, atopy patch test reactions in AE patients (APT-AE), and seborrhea eczema.²⁹ In addition to providing a barrier function, the keratinocytes in skin can secrete numerous cytokines, chemokines, and some growth factors in an autocrine manner that regulates barrier homeostasis, but this ability of keratinocytes has been shown to be deregulated in AE lesional skin.²⁷

The significant drop in serum levels during quiescence not only signifies the potential of APRIL to serve as an efficient marker of disease activity but also signifies the importance of upregulation of APRIL by allergen exposure and microbial products in the initiation and amplification of the atopic skin response. Indeed, this seems to be the case in the local skin environment, where mRNA expression of BLyS is found to dramatically increase in APT-AE, which mimics the acute lesion.^{26,27}

We failed to demonstrate a reduction in serum APRIL levels in our AE patients after the remission of flare of sufficient significance to approach the levels in control subjects. Moreover, a positive correlation between APRIL serum levels during AE flare and corresponding values during remission was elicited. This may reflect the ongoing process of subclinical allergic cutaneous inflammation. It may also indicate the need for more intensive non-conventional anti-inflammatory therapy. Therefore, we suggest that the serum APRIL level can be used as a marker of ongoing inflammatory activity in the skin.

Clinical signs are a core outcome domain in AE trials. The current lack of standardization of outcome measures in AE trials hampers evidence-based communication.³⁰ A wide variety of laboratory measurements have been linked to severity, but most of them lack specificity.³¹ Therefore, the search for an objective marker that may have a more precise basis for the monitoring of such disease is continual.

This study demonstrated that serum APRIL concentrations correlated significantly with the indices of AE severity, namely, the LSS, SSS, and SCORAD indices. In the case of the Objective SCORAD, in which subjective features (pruritus and sleep loss) are omitted, a non-significant correlation was observed. This verifies that

overexpression of APRIL induces pruritus and severe itching and substantiates the importance of subjective symptoms in the evaluation of AE and of the patient's quality of life as pruritus is a major component of AE activity. Therefore, SCORAD may be superior to the Objective SCORAD despite the fact that we initially considered the latter would be more accurate in our patients in view of their age and social background.

Moreover, the Objective SCORAD index differentiated children with severe disease from those with mild-to-moderate disease. This substantiates the usefulness of APRIL as an objective marker of the severity of AE disease. Our results were discordant with those of Chen *et al.*²⁶ This may be ascribed to subject intervariability.

Serum APRIL levels could not be correlated to serum total IgE concentrations or percentage differences from normal during AE flare ($P > 0.05$ for both). In this respect, our results were concordant with those reported by Chen *et al.*²⁶ but not with those of Laske and Niggemann.³² The latter discrepancy could be ascribed to the relatively small number of patients with elevated IgE in our study ($n = 10/50$). We also recruited subjects at different stages of disease activity, and serum IgE levels are known to fluctuate throughout AE stages. It should be noted that AE is not exclusively an IgE-mediated allergic disorder; although a proportion of AE patients have been found to show normal or even decreased total IgE levels, these patients had a non-IgE-associated type of AE syndrome or what was formerly called the intrinsic type of AE.³³ The reasons for these discrepancies are unclear, but they suggest that multiple studies in larger cohorts are required to establish whether levels of these factors really are altered in children with AE.

In the current study, there were no significant correlations between serum APRIL levels and AEC. Previous authors have reported rough correlations between eosinophil counts and disease severity and that very high counts were common in patients with severe AE and a personal or family history of respiratory atopy, whereas normal or moderately elevated counts were obtained in persons with severe or pure AE and no history of respiratory atopy.^{34,35} These findings suggest that disease severity and a history of respiratory atopy are important factors in determining high AEC in AE. As the children recruited in our study had pure AE without any history of other atopic disorders, the lack of correlation between indices of AE severity and AEC was expected.

Serum total LDH levels in AE patients during flare were significantly higher than those in control subjects. Moreover, significant positive correlations were elicited between serum LDH levels during flare and serum APRIL concentrations and the LSS and SCORAD indices of severity. Elevated levels can be attributed to the release of

APRIL from damaged lesional epidermal cells into peripheral blood, which reflects the extent of skin involvement and the degree of epidermal injury. Because LDH is elevated in many other diseases, it has been previously reported as a useful marker for the severity of skin eruption.^{36,37}

The present results lend strong support to the suggestion of an important role of APRIL in the pathogenesis of AE. The serum APRIL level is an objective and reliable marker of the severity of AE in children. APRIL may be a new target in the treatment of AE.

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