

# Serum interleukin 17, interleukin 23, and interleukin 10 values in children with atopic eczema/dermatitis syndrome (AEDS): Association with clinical severity and phenotype

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## ABSTRACT

To date cytokines profile in AEDS is poorly described in children. We evaluated the interleukin (IL)-17, IL-23, and IL-10 levels in atopic eczema/dermatitis syndrome (AEDS) children and healthy controls, in atopic AEDS (aAEDS) and nonatopic (naAEDS) subtypes and their relationship with disease severity. A total of 181 children with aAEDS and 93 healthy children were evaluated. According to the skin-prick test (SPT) for allergens and serum total IgE, all patients were subdivided in two groups: 104 aAEDS and 77 naAEDS. In all patients, serum IL-17, IL-23, and IL-10 levels were detected. Serum IL-17 and IL-23 levels were significantly higher, and serum IL-10 levels were significantly lower in AEDS children than healthy group ( $p < 0.001$ ). Moreover, serum IL-17 and IL-23 levels were significantly higher in aAEDS than in naAEDS subtypes ( $p < 0.001$ ). Differently, serum IL-10 levels resulted similar in both subtypes. There was a correlation between Score Atopic Dermatitis (SCORAD) index and both IL-17 and IL-23 and an inverse correlation between SCORAD index and IL-10 in aAEDS and naAEDS types. Serum IL-17 and IL-23 values were positively related to total IgE levels ( $p < 0.0001$ ) in aAEDS. Further increase of IL-17 and IL-23 levels was detected in aAEDS subjects with atopic diseases such as asthma and rhinitis than children with only allergic sensitization. Our study confirms the role of IL-17, IL-23, and IL-10 and their relationship with the severity of AEDS. We firstly found a correlation between high IL-17/IL-23 axis levels and different phenotypes of AEDS in children, suggesting its role as marker of "atopic march" and disease severity.

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**A**topic eczema/dermatitis syndrome (AEDS) is a chronic relapsing-remitting inflammatory skin disorder beginning mostly in early childhood.<sup>1</sup> AEDS is sustained by a complex interaction between genetics, immunologic, and environmental factors, and it is also characterized by a skin barrier dysfunction resulting in epidermal damage and altered permeability to allergens and microbes.<sup>2</sup> Its prevalence is continuously growing, affecting 15%–30% of children and 2%–10% of the adult population,<sup>3,4</sup> with a relevant socioeconomic burden,<sup>5,6</sup> and requiring an integral allergist/immunologist's training and practice.<sup>7</sup> Pediatric patients with AEDS make up a substantial proportion of patients seen by general pediatricians, allergists, dermatologists, and other specialists. As such, there is a need to optimize understanding and management of

AEDS, and there have been several recent breakthroughs in the diagnosis, pathophysiology, and management of AEDS. Despite these advances, much work is still needed to ensure optimal care for AEDS sufferers.<sup>8</sup> Regarding pathogenesis of AEDS, it is complex and still not fully understood. However, it is also known that the immune system plays a key role in the development, maintenance, and flare-up of AEDS.<sup>2</sup> The disease is characterized mainly by a biphasic T-cell polarization. In the acute phase, the lesions are characterized by an infiltrating Th2 cells that produce interleukin (IL)-4, promoting Th2 cells to become refractory to regulatory T cell suppression,<sup>9</sup> IL-5, playing an important role in disease dysregulation,<sup>9</sup> and IL-13. In the chronic phase, there is a switch toward a Th1 phenotype producing interferon- $\gamma$ , favoring skin infections,<sup>9</sup> and IL-2, and inhibiting the release of IL-4.<sup>10–12</sup> Thus, both cellular and humoral components can dysregulate immune response in patients with AEDS, also enhancing IgE synthesis.<sup>13</sup> In addition to traditional Th2/Th1 cytokines, recently discovered proinflammatory Th2 (e.g., IL-25, IL-31, IL-33, and IL-9) and Th22 (e.g., IL-22, IL-13, and fibroblast growth factor cytokines) also participate in AEDS, contributing to the amplification of the immune response by enhancing the interleukines

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and chemokines released by several cellular subsets, supporting progressive activation of Th2/Th22 axis from acute to chronic phases.<sup>14</sup> Thus, some authors demonstrate that recently discovered Th2 cytokines, especially IL-31, are strongly related to the severity of the inflammatory process. Accordingly, it has been also reported that IL-31 is directly proportional to Score Atopic Dermatitis (SCORAD) and serum levels of IL-4 and IL-13.<sup>15</sup>

Depending on the association or not to IgE sensitization, AEDS may be defined as atopic (aAEDS) or nonatopic (naAEDS). The aAEDS is the most prevalent form of the disease, and it affects approximately 70%–85% of patients. These patients have high serum IgE levels, positive skin-prick test (SPT) reactions to common environmental allergens such as foods and aeroallergens, and sometimes concomitant atopic diseases such as asthma and/or rhinitis.<sup>16,17</sup> The naAEDS is associated with no sensitization to foods or aeroallergens, normal serum IgE values, negative SPT, and no atopic disease. Nevertheless, these patients manifest identical skin lesions of children with atopic AEDS that in 30%–60% of cases will develop allergic respiratory disease,<sup>18</sup> whereas children with naAEDS rarely will go on to develop asthma.<sup>19,20</sup>

Because it seems that children with naAEDS develop SPT positivity to epicutaneous tests to allergens within a decade,<sup>19</sup> it has been even suggested that this latter form represents a transitional step of the natural history of atopic dermatitis,<sup>21</sup> especially in the light of the similar morphologic and distribution of skin involvement.<sup>22</sup>

These findings confirm that the pathogenesis of the disease is still less clear<sup>21,23–26</sup> and that many different pathways may be involved.

To date, besides Th1 and Th2 cells, other T-cell subsets, including T regulatory cells (Th-reg), and Th17 cells seem to have a role in the pathogenesis of AEDS.<sup>27,28</sup>

Th17 subsets produce some of proinflammatory cytokines such as IL-21, IL-22, tumor necrosis factors- $\gamma$ , and IL-17.<sup>29</sup> IL-17 plays a crucial role in the pathophysiology of AEDS<sup>30</sup> because it is highly detectable in acute AEDS and during the relapses of the disease.<sup>31</sup>

Cytokine production by Th17 cells is even promoted by IL-23, produced by the innate immune system and by dendritic cells in response to trigger factors that favor the maintenance of chronic skin inflammatory condition.<sup>32,33</sup> Additionally, both IL-17 and IL-23 stimulate further Th17 cells, maintaining the inflammatory response to tissue injury.

IL-10, mainly produced by Th-reg, is a suppressive cytokine of T-cell proliferation in both Th1 and Th2 cells, and it seems to be an important immune factor to regulate the immune response, and it favors the immune tolerance.<sup>33</sup> Data from studies examining IL-10

in subjects with AEDS are conflicting, because lower values were found in severe AEDS patients than mild AEDS patients and/or healthy controls. In contrast to these findings, other studies have shown elevated IL-10 levels in serum and in skin of AEDS subjects.<sup>34–37</sup> Elevated number of CD4<sup>+</sup> and CD8<sup>+</sup> cells expressing IL-10 was also observed in subjects with AEDS.<sup>38</sup> Moreover, it is necessary to remember the critical role of CD62 ligand on naive and memory T cells, which might serve as a predictive marker for AEDS with other overlapping atopic disorders.<sup>39</sup>

Because data on serum IL-10, IL-17, and IL-23 in AEDS patients are limited, we investigated their serum levels in AEDS children and compared their levels in the atopic and nonatopic form of the disease. In addition, we assessed the relationship between their levels and clinical severity of the disease.

## PATIENTS AND METHODS

The present study evaluated 181 children (82 females, 99 males; median age was  $8.31 \pm 3.21$  years) with AEDS and 93 healthy children (42 females, 51 males; median age was  $8.51 \pm 2.93$  years).

Children were recruited at the Pediatric Department of the University Hospital of Messina, Italy. Institutional Review Board approved the study. All the participating subjects were from Italian origin. AEDS was diagnosed and assessed according to validated criteria,<sup>40</sup> clinical grading of skin severity was defined according to SCORAD index of the European Task Force on Atopic Dermatitis.<sup>41</sup>

Depending on total IgE levels and SPT, children with AEDS were divided into atopic group (aAEDS: 104 children, 55 males and 49 females; aged  $8.7 \pm 1.89$  years) and nonatopic group (naAEDS: 77 children, 44 males and 33 females; aged  $9.01 \pm 2.2$  years). Furthermore, among 104 aAEDS patients, 50 subjects showed concomitant atopic diseases such as asthma and/or rhinitis and 54 only allergic sensitization.

aAEDS status was defined as having high levels of total IgE and at least one positive SPT. naAEDS patients had normal blood total IgE levels and negative response to a prick test to common aero- and foods allergens.

SPT was performed on the volar aspect of the lower arms by using extracts for food- (egg yolk, egg white, cow's milk, peanuts, fruits, soya, and hazelnut) and aeroallergens [cat and dog dander, dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), ragweed, mixed trees, mixed grasses, and molds (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* species; Lofarma, S.p.A., Milano, Italy)]. Skin wheal diameter was recorded at 15 minutes as the mean of two perpendicular measurements. We used histamine 10 mg/mL as the positive control and a 0.9% saline as

Table 1. Total IgE , IL-17, IL-23, and IL-10 in AEDS subjects and healthy controls

|                 | AEDS Patients  | Healthy Controls | <i>p</i> -Value |
|-----------------|----------------|------------------|-----------------|
| N               | 181            | 93               |                 |
| Total IgE UI/mL | 215.25 ± 18.81 | 73.4 ± 20.3      | <0.001          |
| IL-17 pg/mL     | 63.21 ± 5.47   | 29.3 ± 12.1      | <0.001          |
| IL-23 pg/mL     | 211.93 ± 38.41 | 111.3 ± 13.4     | <0.001          |
| IL-10 pg/mL     | 3.21 ± 0.55    | 4.95 ± 0.5       | <0.001          |

AEDS = atopic eczema/dermatitis syndrome; IL = interleukin.

the negative control. A positive response was defined as a skin wheal diameter of three millimeters or more compared with negative control.<sup>42</sup> Exclusion criteria included: other dermatological illnesses, previous use of systemic corticosteroids or immunosuppressive therapy, topical corticosteroids and antihistamines were stopped for at least four weeks before inclusion in the study.

Of the 104 children with aAEDs, 32 (30.8%) were assessed to have mild disease, 37 (35.6%) to have moderate disease, and 35 (33.6%) to have severe disease. Nearly 48% of children (n = 50) with positive SPT had concomitant asthma and/or rhinitis. Sensitization to inhalant allergens was relatively common (76%) as compared with food allergens (24%). House dust mite (37%), parietaria (14.3%) and graminaceae (11.8%) were the most common positive reactions.

Of the 77 children with naAEDS, 25 (32.5%) had mild disease, 29 (37.6%) moderate disease, and 23 (29.9%) severe disease.

The control group comprised healthy children without past or current history of allergic diseases and dermatological illnesses, normal peripheral blood eosinophils count, and normal serum IgE levels.

The study was explained in detail to the parents/guardians of the children who participated in this study, and an informed consent was obtained from each parent/guardian. A physician performed clinical examination in all children. Demographic data and other clinical characteristics of the study subjects were collected.

All subjects were subjected to blood sampling. The blood samples from the healthy controls and AEDS subjects were collected by venipuncture at appropriate conditions. Serum was acquired after coagulation of the blood for 1–2 hours at room temperature. The supernatant was centrifuged for 10 minutes at 2000 × g. The serum thus acquired was then stored at –70°C.

#### Assay of Serum Cytokines

IL-17, IL-23, and IL-10 enzyme-linked immunosorbent assay kits were used by a solid phase enzyme-amplified sensitivity immunoassay method on microtiter plate. The assays used monoclonal antibodies

direct against distinct epitope of IL-17, IL-23, and IL-10. Standards (78–5000 pg/mL) and samples reacted with the capture monoclonal antibody coated on microtiter well, a monoclonal antibody labeled with biotin and with a monoclonal antibody labeled with horseradish peroxidase. After an incubation period allowing the formation of a sandwich, coated-human IL-17/23/10-biotin-horseradish peroxidase, the microtiter plate was washed to remove unbound enzyme-labeled antibody. Bound enzyme-labeled antibody was measured through a chromogenic reaction. Chromogenic solution (tetramethylbenzidine) was added and incubated for 15 minutes. The reaction was stopped with the addition of Stop Solution, and the microtiterplate was successively read at the 450-nm wavelength. The amount of substrate turnover was determined colorimetrically by measuring the absorbance, which was proportional to the IL-s concentration. A calibration curve was plotted, and IL-s concentration in samples was determined by interpolation from the calibration curve. The use of enzyme-amplified sensitivity immunoassay reader (linearity up to three optical density units) and sophisticated data reduction method (polychromatic data reduction) resulted in a high sensitivity at the low range and in an extended calibration range.

#### Statistical Analysis

The data collected were statistically analyzed by the statistical computer software SPSS, version 15.0. A *p*-value of less than 0.05 was considered to be statistically significant. Statistical analysis was performed with ANOVA test and relatives post hoc versus control. Mean and standard deviation were calculated for the variables, and *t*-test was employed for the comparisons. For the attributes, percentages were calculated first, and then *v2* test was used for comparisons. Correlations between IL-17, IL-23, and IL-10 levels and SCORAD index and between serum total IgE levels and serum IL-17, IL-23, and IL-10 levels were calculated using Spearman's correlation coefficient.

#### RESULTS

The data characteristics of the patients and healthy controls are presented in Tables 1 and 2.

Table 2. Total IgE, IL-17, IL-23, and IL-10 in aAEDS, naAEDS subjects, and healthy controls

|               | N   | SCORAD       | Total IgE UI/mL | IL-17 pg/mL   | IL-23 pg/mL    | IL-10 pg/mL  |
|---------------|-----|--------------|-----------------|---------------|----------------|--------------|
| aAEDS         | 104 |              | 360.7 ± 25.34   | 72.49 ± 10.00 | 235.84 ± 65.07 | 3.28 ± 0.89  |
| Mild          | 32  | 20.31 ± 2.23 | 333.24 ± 23.59  | 59.30 ± 3.219 | 180.57 ± 12.25 | 3.88 ± 0.48  |
| Moderate      | 37  | 9.76 ± 9.035 | 366.02 ± 7.673  | 74.08 ± 2.54  | 233.67 ± 13.20 | 3.678 ± 0.50 |
| Severe        | 35  | 6.67 ± 4.52  | 85.25 ± 6.42    | 82.87 ± 3.07  | 288.965 ± 9.58 | 2.19 ± 0.25  |
| naAEDS        | 77  |              | 69.80 ± 12.29   | 53.94 ± 0.95  | 188.02 ± 11.75 | 3.15 ± 0.22  |
| Mild          | 25  | 21.33 ± 2.13 | 68.37 ± 11.46   | 50.01 ± 0.86  | 148.66 ± 8.15  | 3.91 ± 0.23  |
| Moderate      | 29  | 7.21 ± 8.645 | 67.75 ± 15.45   | 53.86 ± 0.75  | 190.12 ± 14.8  | 3.63 ± 0.20  |
| Severe        | 23  | 4.31 ± 2.11  | 73.3 ± 9.7      | 57.97 ± 1.25  | 225.3 ± 12.3   | 1.93 ± 0.24  |
| Control group | 93  | —            | 73.4 ± 20.3     | 29.3 ± 12.1   | 111.3 ± 13.4   | 4.95 ± 0.5   |

AEDS = atopic eczema/dermatitis syndrome; aAEDS = atopic AEDS; IL = interleukin; naAEDS = nonatopic AEDS; SCORAD = Score Atopic Dermatitis.

### IL-17

The serum levels of IL-17 were significantly higher in children with AEDS as compared with healthy control group ( $63.21 \pm 5.47$  versus  $29.3 \pm 12$ , respectively) (Table 1).

Furthermore, serum levels of IL-17 were significantly higher in patients with aAEDS than those with naAEDS ( $p < 0.001$ ,  $\chi^2$  value 38,302) (Table 2). Moreover, clinical severity scores correlated significantly with IL-17 levels, in fact, the highest levels of IL-17 were found in the group of children with severe symptoms, and there was a significant difference within AEDS subtypes (Fig. 1).

Finally, the mean serum IL-17 levels were significantly higher in aAEDS with allergic disease compared with sensitive aAEDS subtype without allergic disorders ( $79.83 \pm 4.66$  versus  $64.50 \pm 14$  pg/mL;  $p < 0.0001$ ) (Table 3). Serum IL-17 values were also positively related to serum total IgE levels in allergic disease-aAEDS and sensitive-aAEDS subtype ( $r = 0.819$  vs  $r = 0.7114$ , respectively) (Fig. 2).

### IL-23

The serum levels of IL-23 were significantly higher in children with AEDS as compared with healthy control group ( $211.93 \pm 38.41$  versus  $111.3 \pm 13.4$  pg/mL, respectively;  $p < 0.001$ ) (Table 1). Moreover, the serum levels of IL-23 were significantly higher in patients with aAEDS than those with naAEDS ( $p < 0.001$ ,  $\chi^2$  value 79.62) (Table 2).

Clinical severity scores correlated significantly with IL-23 levels, in fact, the highest levels of IL-23 were found in the group of children with severe symptoms, and there was a significant difference within AEDS subtypes (Fig. 1). Moreover, the mean serum level of IL-23 was significantly higher in allergic disease-aAEDS subtype compared with sensitive-aAEDS subtype ( $270.53 \pm 45.11$  versus  $193.10 \pm 52.67$  pg/mL;  $p < 0.0001$ ) (Table 3). Finally, serum IL-23 levels were

positively to plasma total IgE levels in allergic disease-aAEDS and sensitive-aAEDS subtype ( $r = 0.78$  vs  $r = 0.88$ , respectively) (Table 3 and Fig. 2).

### IL-10

The serum levels of IL-10 were lower in children with AEDS as compared with healthy control group ( $3.21 \pm 0.55$  pg/mL, respectively, versus  $4.95 \pm 0.5$  pg/mL;  $p < 0.001$ ) (Table 1).

Serum IL-10 levels showed a significantly inverse correlation with severity scores, in fact, IL-10 levels were significantly lower in children with aAEDS as compared with healthy control group, and the lowest levels were found in the group of children with severe symptoms (Fig. 1). We have found no significant differences between serum levels of IL-10 in children with aAEDS respect to those with naAEDS ( $p > 0.05$ ).

### Total IgE

Serum total IgE levels were significantly increased in the subjects with aAEDS ( $360.7 \pm 25.34$  U/mL) in comparison with naAEDS ( $69.80 \pm 12.29$  U/mL) and healthy children ( $73.4 \pm 20.3$  U/mL) ( $p < 0.001$ ). Total IgE levels significantly related with cytokines in children with aAEDS and related subtypes: positively with IL-17 and IL-23 but negatively with IL-10. In addition, the mean serum total IgE levels were significantly higher in allergic disease aAEDS subtype compared with sensitive-aAEDS subtype ( $p < 0.0001$ ) (Table 3 and Fig. 2).

### DISCUSSION

Atopic eczema dermatitis syndrome is a chronic relapsing-remitting inflammatory skin disorder characterized by an impaired immune response beginning mostly in early childhood.

It is considered a polar Th2 disease in acute phase, with a partial shift to Th1 during the chronic phase,<sup>10,11</sup> in

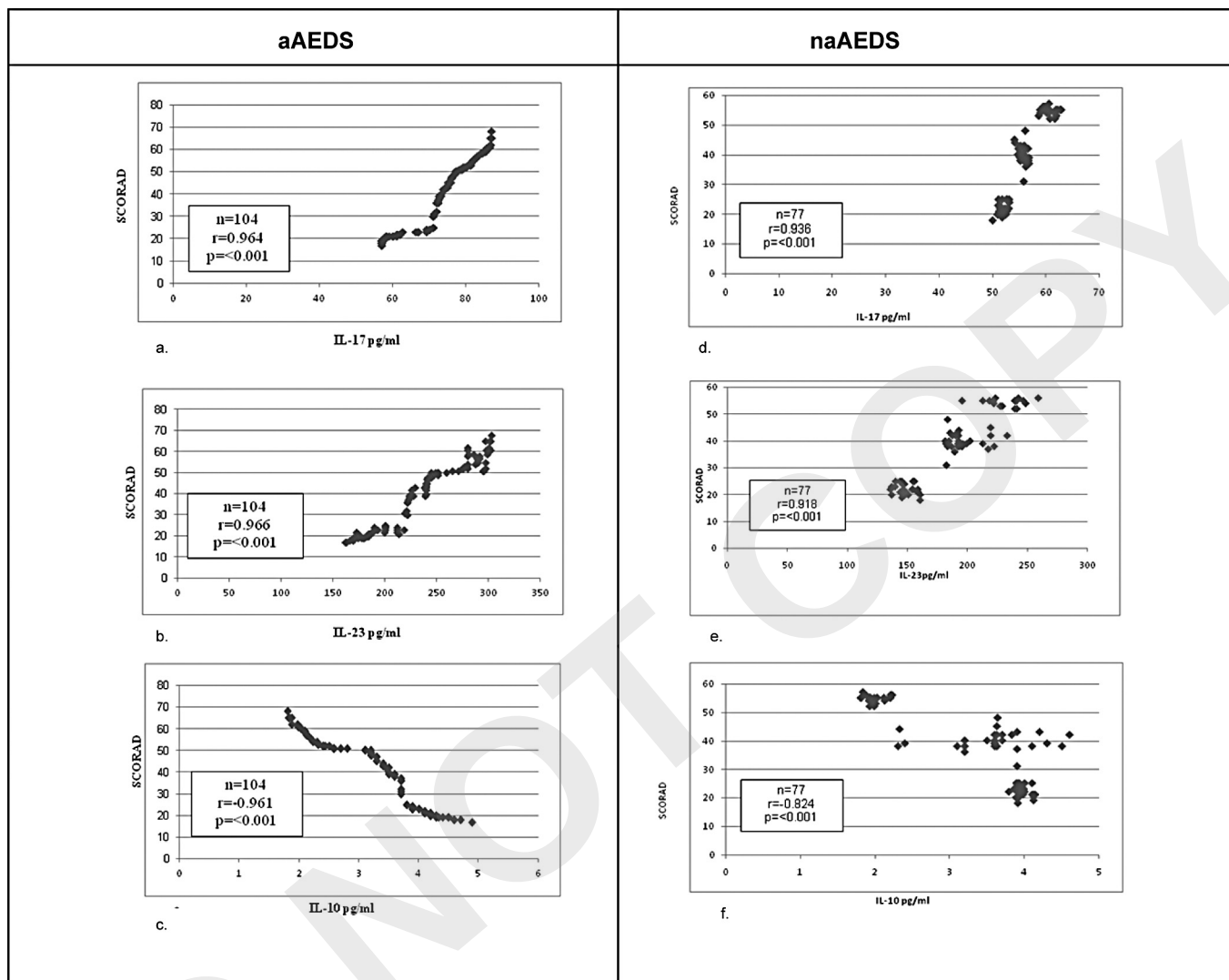


Figure 1. Correlations between serum IL-17 (A and D), IL-23 (B and E), IL-10 (C and F) levels (pg /mL) and SCORAD index in children with aAEDS and naAEDS.

Table 3. Total IgE, IL-17, and IL-23 in aAEDS subtypes

|             | aAEDS                                    |                                             | p/r     |
|-------------|------------------------------------------|---------------------------------------------|---------|
|             | Positive SPT with asthma and/or rhinitis | Positive SPT without asthma and/or rhinitis |         |
| N           | 54                                       | 50                                          |         |
| IL-17 pg/mL | 64.50 ± 14                               | 79.83 ± 4.66                                | <0.0001 |
| IL-23 pg/mL | 193.10 ± 52.67                           | 270.53 ± 45.11                              | <0.0001 |
| IL-10 pg/mL | 3.11 ± 1.6                               | 3.02 ± 1.63                                 | >0.05   |

aAEDS = atopic atopic eczema/dermatitis syndrome; IL = interleukin; p = p-value; r = Pearson's r correlation; SPT = skin-prick test.

which innate and adaptive immune system plays complex interactions, which are triggered by genetic, including family atopy<sup>43,44</sup> and environmental factors.<sup>45</sup>

Pathogenic role for Th1 and Th2 cells has been demonstrated,<sup>11</sup> but the classic Th1/Th2 paradigm

previously defining AEDS has been recently challenged.

In fact, other T-cells subsets, including proinflammatory cells like Th9, Th17, and Th22, expressing high levels of IL-9, IL-17, and IL-22 together with the anti-

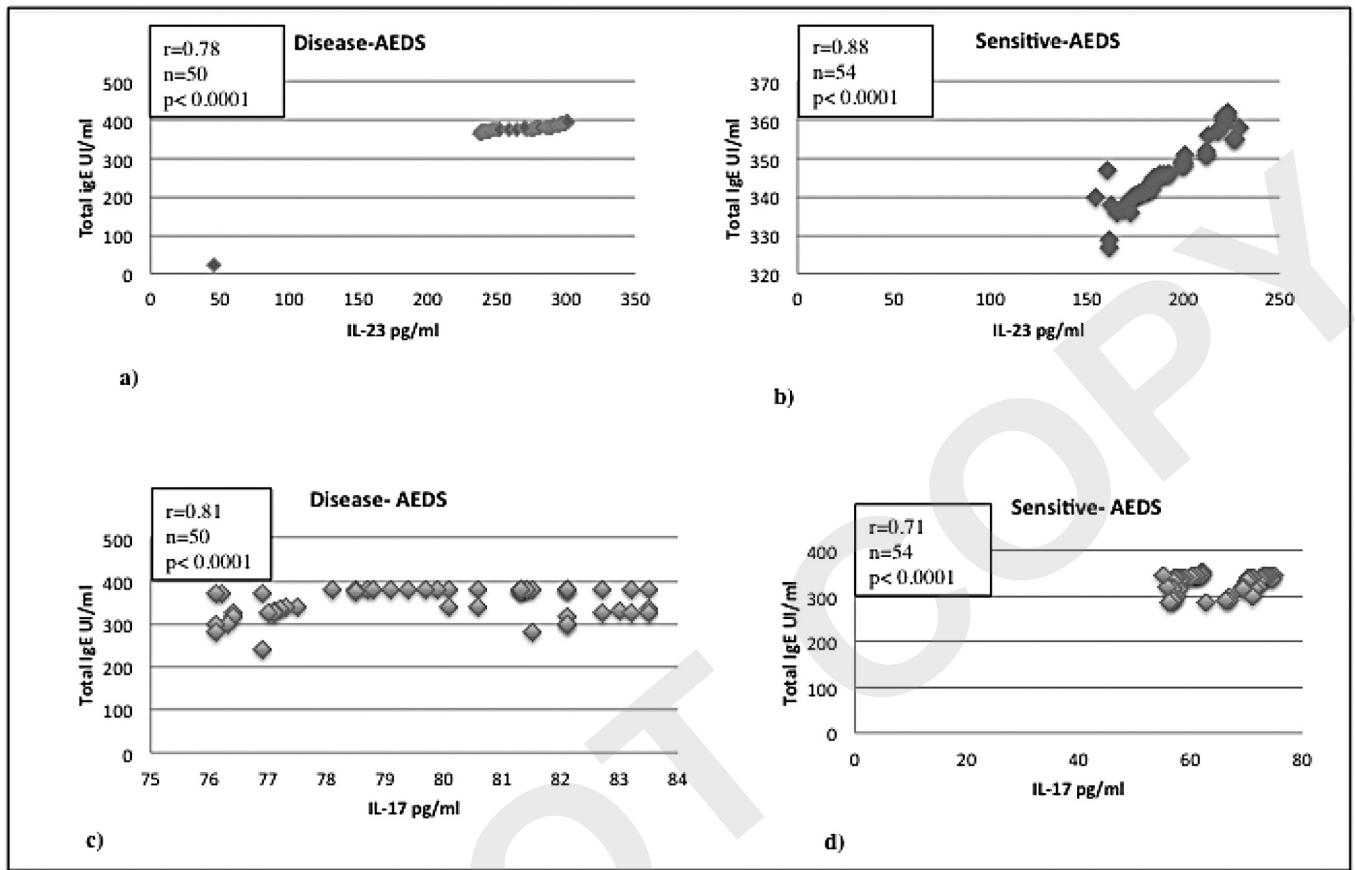


Figure 2. Correlation between serum IL-23 (A and B) and IL-17 (C and D) levels and serum total IgE levels in aAEDS subtypes.

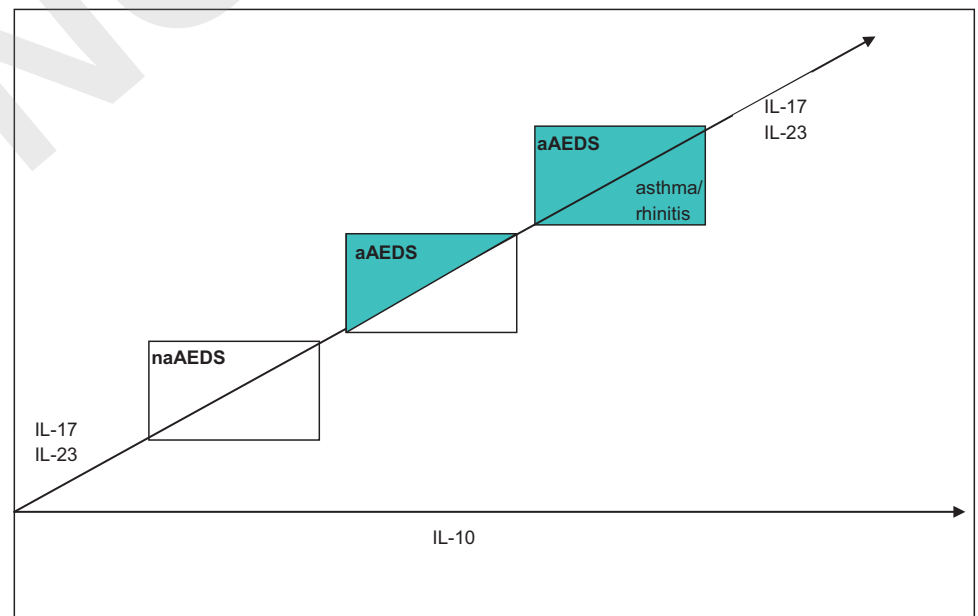


Figure 3. IL-17/IL-23 axis and "allergic march."

inflammatory and immune-modulating Th-reg cells constitutively producing IL-10, have been widely studied with conflicting results.<sup>27,28</sup>

In this study, we examined the levels of IL-17, IL-23, and IL-10 in children with AEDS. In particular, the cur-

rent study was designed to investigate their levels in atopic and nonatopic AEDS and the presence of a relationship between these cytokines levels and disease severity.

It is known that IL-17, characteristically produced by Th17 cells, is a key cytokine in host defense infections

as well in autoimmune diseases.<sup>46</sup> It plays a key role in the pathophysiology of AEDS,<sup>30</sup> because is highly detectable in acute AEDS and during the relapse of chronic stage of disease<sup>31</sup> while being barely expressed in the stable chronic phase of the disease.<sup>47</sup>

Also, IL-23 may be an adjunctive Th17-associated cytokine involved in the worsening of inflammatory phenomena, because it maintains the chronic skin inflammatory condition.<sup>32</sup>

IL-23 is produced by the innate immune system and by dendritic cells in response to trigger factors, and it is a novel member of the IL-12 family, which synergizes with IL-6 and IL-1 to promote Th17 expansion and production.<sup>32</sup>

Our results show that both serum IL-17 and IL-23 levels are significantly elevated in children with AEDS especially in patients with more severe disease. Moreover, we found a direct correlation between IgE values and both IL-17 and IL-23 and significant higher values of both IL-17 and IL-23 in aAEDS with associated atopic diseases such as asthma and/or rhinitis than in aAEDS with only allergic sensitization or in naAEDS. We suppose that both IL-17 and IL-23, influencing Th2 pattern, might modulate allergic inflammation also in AEDS. In this regard, it has been initially reported in literature that serum IL-23 levels were significantly higher in children with asthma than in healthy well-matched controls,<sup>48–50</sup> and our findings seem to confirm this result also in AEDS patients.

IL-10 is an antiinflammatory cytokine produced mainly by T-reg cells. Although it is well known that IL-10 down-regulates the immune system minimizing tissue damage during inflammation,<sup>51</sup> data regarding its role in AEDS subjects are conflicting.

In fact, authors have shown increased serum IL-10 levels in peripheral blood mononuclear cells<sup>52</sup> and in lesional skin<sup>53</sup> from AEDS patients. Conversely, other studies reported that serum IL-10 levels inversely correlate with the severity of AEDS<sup>54</sup> and, in according to these data, AEDS exacerbations have been associated with decreased IL-10 function.<sup>53</sup> Confirming these results, in our study, we found significant lower serum IL-10 values in AEDS patients than healthy controls. Moreover, we found an inverse relationship between IL-10 levels and severity of the AEDS.

Differently from IL-17 and IL-23, we found no difference of IL-10 between the aAEDS and naAEDS subtypes and no correlation with IgE values. These data suggest that reduced IL-10 levels in AEDS children are related to skin inflammation independently by AEDS subtype. Because it is known that IL-10 regulates the production of IL-17, we hypothesized that low levels of IL-10 in AEDS patients might function physiologically to dampen IL-17 production in both subtypes of aAEDS and naAEDS patients.

To our knowledge, this study is the first to find a clear correlation between high IL-17/IL-23 axis levels and phenotypes of AEDS in children. We hypothesized that IL-17/IL-23 axis is relevant in the pathogenesis of the disease, and it could be measured as a possible marker of “atopic march”<sup>55</sup> and disease severity in AEDS patients, according to their age and their course of eczema (Fig. 3).

Moreover, these underlying mechanisms regarding IL-17/IL-23 axis and IL-10 might represent the link between atopic and nonatopic disease.<sup>19,56,57</sup>

Our results confirm the complexity of the inflammatory regulation of this unique atopic disease, although further studies are clearly needed to give deeper insight into the significant role of these cytokines in the pathogenesis of disease.

The present data might also have clinical relevance to evaluate whether therapeutic strategies for the treatment of AEDS are capable of diminishing IL-17 and IL-23 production and of increasing IL-10 production.

## REFERENCES

1. Leung DY, Boguniewicz M, and Howell MD. New insights into atopic dermatitis. *J Clin Invest* 113:651–657, 2004.
2. Shaker M. New insights into the allergic march. *Curr Opin Pediatr* 26:516–520, 2014.
3. Braback L, Hjert A, and Rasmussen F. Trends in asthma, allergic rhinitis and eczema among Swedish conscripts from farming and non-farming environments. A nationwide study over three decades. *Clin Exp Allergy* 34:38–43, 2004.
4. DaVeiga SP. Epidemiology of atopic dermatitis: a review. *Allergy Asthma Proc* 33:227–234, 2012.
5. Bieber T. Atopic dermatitis. *N Engl J Med* 358:1483–1494, 2008.
6. Leung DY, and Bieber T. Atopic dermatitis. *Lancet* 361:151–160, 2003.
7. Lio PA, Lee M, LeBovidge J, et al. Clinical management of atopic dermatitis: practical highlights and updates from the atopic dermatitis practice parameter 2012. *J Allergy Clin Immunol Pract* 2:361–369; quiz 370, 2014.
8. Totri CR, Diaz L, Eichenfield LF. 2014 update on atopic dermatitis in children. *Curr Opin Pediatr* 26:466–471, 2014.
9. Koulouri M, Vrachnou E, Liatsis E, et al. PD36 - Evaluation of intracellular cytokines IL-2, IFN $\gamma$ , IL-4 and IL-5 in children with atopic dermatitis and correlations with other immunological and epidemiological parameters. *Clin Transl Allergy* 28:4, 2014.
10. Ong PY, and Leung DY. Immune dysregulation in atopic dermatitis. *Curr Allergy Asthma Rep* 6:384–389, 2006.
11. Grewe M, Bruijnzeel-Koomen CA, Schöpf E, et al. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 19:359–361, 1998.
12. Frieri M, Martinez S, Agarwal K, and Trotta P. A preliminary study of interleukin-4 detection in atopic pediatric and young adult patients. *Pediatr Asthma Allergy Immunol* 7:27–35, 1993.
13. Knapik M, and Frieri M. Altered cytokine production in atopic dermatitis. *Pediatr Asthma Allergy Immunol* 7:127–133, 1993.
14. Peng W, and Novak N. Recent developments in atopic dermatitis. *Curr Opin Allergy Clin Immunol* 14:417–22, 2014.
15. Raap U, Weißmantel S, Gehring M, et al. IL-31 significantly correlates with disease activity and Th2 cytokine levels in children with atopic dermatitis. *Pediatr Allergy Immunol* 23:285–288, 2012.
16. Schmid-Grendelmeier P, Simon D, and Simon HU. Epidemiology, clinical features, and immunology of the “intrinsic” (non-

- IgE-mediated) type of atopic dermatitis (constitutional dermatitis). *Allergy* 56:841–849, 2001.
17. Havstad S, Johnson CC, Kim H, et al. Atopic phenotypes identified with latent class analyses at age 2 years. *J Allergy Clin Immunol* 134:722–727.e2, 2014.
18. ETAC STUDY GROUP. Allergic factors associated with the development of asthma and the influence of cetirizine in a double-blind, randomised, placebo-controlled trial: first results of ETAC. Early Treatment of the Atopic Child. *Pediatr Allergy Immunol* 9:116–124, 1998.
19. Novembre E, Cianferoni A, Lombardi E, et al. Natural history of “intrinsic” atopic dermatitis. *Allergy* 56:452–453, 2001.
20. Wüthrich B, and Schmid-Grendelmeier P. Natural course of AEDS. *Allergy* 57:267–268, 2002.
21. Novak N, and Bieber T. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol* 112:252–262, 2003.
22. Aquino M, and Fonacier L. The role of contact dermatitis in patients with atopic dermatitis. *J Allergy Clin Immunol Pract* 2:382–387, 2014.
23. Leonardi S, Miraglia del Giudice M, and La Rosa M. Atopic disease, immune system, and the environment. *Allergy Asthma Proc* 28:410–417, 2007.
24. Miraglia del Giudice M, Decimo F, and Leonardi S. Immune dysregulation in atopic dermatitis. *Allergy Asthma Proc* 27:451–455, 2006.
25. Leonardi S, Rotolo N, and Vitaliti G. IgE values and T-lymphocyte subsets in children with atopic eczema/dermatitis syndrome. *Allergy Asthma Proc* 28:529–534, 2007.
26. Hogan AD, and Burks AW. Epidermal Langerhans cells and their function in the skin immune system. *Ann Allergy Asthma Immunol* 71:5–10, 1995.
27. Souwer Y, Szegedi K, and Kapsenberg ML. IL-17 and IL-22 in atopic allergic disease. *Curr Opin Immunol* 22:821–826, 2010.
28. Ciprandi G, De Amici M, Giunta V, et al. Serum interleukin-9 levels are associated with clinical severity in children with atopic dermatitis. *Pediatr Dermatol* 30:222–225, 2013.
29. Koga C, Kabashima K, Shiraishi N, et al. Possible pathogenic role of Th17 cells for atopic dermatitis. *J Invest Dermatol* 128:2625–2630, 2008.
30. Souwer Y, Szegedi K, Kapsenberg ML, and de Jong EC. IL-17 and IL-22 in atopic allergic disease. *Curr Opin Immunol* 22:821–826, 2010.
31. Toda M, Leung DY, Molet S, et al. Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol* 111:875–881, 2003.
32. Kastelein RA, Hunter CA, and Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 25:221–242, 2007.
33. Couper KN, Blount DG, and Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 180:5771–5777, 2008.
34. Simon D, Braathen LR, and Simon HU. Increased lipopolysaccharide-induced tumour necrosis factor- $\alpha$ , interferon- $\gamma$  and interleukin-10 production in atopic dermatitis. *Br J Dermatol* 157:583–586, 2007.
35. Asadullah K, Sterry W, and Stephanek K. IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach. *J Clin Invest* 101:783–794, 1998.
36. Howell MD, Novak N, Bieber T, et al. Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. *J Invest Dermatol* 125:738–745, 2005.
37. Ohmen JD, Hanifin JM, and Nickoloff BJ. Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J Immunol* 154:1956–1963, 1995.
38. Aleksza M, Lukács A, Antal-Szalmás P, et al. Increased frequency of intracellular interleukin (IL)-13 and IL-10, but not IL-4, expressing CD4+ and CD8+ peripheral T cells of patients with atopic dermatitis. *Br J Dermatol* 147:1135–1141, 2002.
39. Frieri M, Bennuri B, Dias C, et al. Association between CD62 ligand on naïve and memory T cells and history of cow’s milk hypersensitivity in atopic patients. *Ann Allergy Asthma Immunol* 92:565–572, 2004.
40. Rajka G, and Langeland T. Grading of severity of atopic dermatitis. *Acta Derm Venerol* 144:13–14, 1989.
41. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 186:23–31, 1993.
42. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. *Clin Transl Allergy* 3:3, 2013.
43. Salpietro C, Rigoli L, Miraglia Del Giudice M, et al. Int J TLR2 and TLR4 gene polymorphisms and atopic dermatitis in Italian children: a multicenter study. *Immunopathol Pharmacol* 24:33–40, 2011.
44. Fuiano N, Delyecchio M, and Incorvaia C. Factors associated with different results of allergy tests in children with dust mite-induced atopic dermatitis. *Allergol Immunopathol (Madr)* S0301–0546(14)00093–7, 2014.
45. Banerjee S, Resch Y, Chen KW, et al. Der p 11 is a major allergen for house dust mite allergic patients suffering from atopic dermatitis. *J Invest Dermatol* 2014. doi: 10.1038/jid.2014.271. [Epub ahead of print]
46. Zhu S, and Qian Y. 17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential. *Clin Sci (Lond)* 122:487–511, 2012.
47. Nograles KE, Zaba LC, Shemer A, et al. IL-22-producing “T22” T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol* 123:1244–1252, 2009.
48. Ciprandi G, Cuppari C, and Salpietro A. Serum IL-23 strongly and inversely correlates with FEV1 in asthmatic children. *Int Arch Allergy Immunol* 159:183–186, 2012.
49. Ciprandi G, Cuppari C, and Salpietro C. Serum IL-23: a surrogate biomarker for asthma? *Clin Exp Allergy* 42:1416–1417; author reply 1418, 2012.
50. Vitaliti G, Leonardi S, Miraglia Del Giudice M, et al. Mucosal immunity and sublingual immunotherapy in respiratory disorders. *J Biol Regul Homeost Agents* 26:85–93, 2012.
51. Sabat R, Grütz G, Warszawska K, et al. Biology of interleukin-10. *Cytokine Growth Factor Rev* 21:331–344, 2010.
52. Simon D, Braathen LR, and Simon HU. Increased lipopolysaccharide-induced tumour necrosis factor- $\alpha$ , interferon- $\gamma$  and interleukin-10 production in atopic dermatitis. *Br J Dermatol* 157:583–586, 2007.
53. Darling AR, Freyschmidt EJ, Burton OT, et al. L-10 suppresses IL-17-mediated dermal inflammation and reduces the systemic burden of Vaccinia virus in a mouse model of eczema vaccinatum. *Clin Immunol* 150:153–60, 2014.
54. Niwa Y. Elevated RANTES levels in plasma or skin and decreased plasma IL-10 levels in subsets of patients with severe atopic dermatitis. *Arch Dermatol* 136:125–126, 2000.
55. Shen CY, Lin MC, Lin HK, et al. The natural course of eczema from birth to age 7 years and the association with asthma and allergic rhinitis: a population-based birth cohort study. *Allergy Asthma Proc* 34:78–83, 2013.
56. Wollenberg A, and Bieber T. Atopic dermatitis: from the genes to skin lesions. *Allergy* 55:205–213, 2000.
57. Cuppari C, Leonardi S, Manti S, et al. Allergen immunotherapy, routes of administration and cytokine networks: an update. *Immunotherapy* 6:775–786, 2014. □

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