

Correlation of skin barrier impairment in atopic dermatitis with aeroallergen sensitization

Federica De Marchi, M.D.,¹ Giorgio L. Piacentini, M.D.,¹ Michele Piazza, B.Sc.,¹ Marco Sandri, Stat.,¹ Attilio L. Boner, M.D.,¹ and Diego G. Peroni, M.D.²

ABSTRACT

Background: Atopic dermatitis (AD) often predates the development of allergic sensitization in the so-called atopic march. Several studies have pointed out epidermal barrier impairment as a major cause of this evolution.

Objective: The present study aimed to assess atopic skin integrity by means of transepidermal water loss (TEWL) and Corneometer, and to investigate possible correlations between barrier integrity measurements and the degree of sensitization to aeroallergens (allergy score).

Methods: Sixty-one children (6 months to 17 years old) with AD were clinically evaluated by the Scoring Atopic Dermatitis index. TEWL and Corneometer evaluations were performed on lesion sites as well as on healthy skin. The subjects underwent skin-prick testing, and the severity of allergic sensitization was assessed for each patient by summing all wheal diameters (the allergy score). The same tests were performed in 20 children without AD.

Results: In patients with AD, TEWL and Corneometer results were found to be higher and lower, respectively, on eczematous areas in comparison with healthy skin, and differences were significantly correlated to the Scoring Atopic Dermatitis index ($p < 0.0001$ and $p = 0.007$, respectively). The TEWL result was significantly higher in nonlesional skin of the patients with AD compared with that of individuals without AD ($p = 0.017$). Of the patients with AD, 59% were sensitized to inhalant allergens; allergy scores were positively correlated with both AD duration ($r = 0.63$; $p < 0.0001$) and nonlesional skin TEWL values ($r = 0.46$; $p = 0.002$). No significant correlation was found between allergy scores and skin parameters in subjects without AD.

Conclusion: Patients with AD are affected by barrier function impairment, even on noneczematous skin. This defect is associated with greater aeroallergen sensitization and may contribute to allergic respiratory symptom development.

(Allergy Asthma Proc 36:e127–e133, 2015; doi: 10.2500/aap.2015.36.3872)

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease, with the highest prevalence rates in childhood.¹ Despite its favorable prognosis, with most patients outgrowing the condition by early adolescence,² in ~40% of patients, AD constitutes the first step toward the development of allergic respiratory symptoms, such as asthma and/or allergic rhinitis.^{3,4} A specific cytokine expression pattern has recently been suggested as a marker of progression of the “atopic march,”⁵ but underlying mechanisms for this process remain unclear. However, many studies in the past decades emphasize the causative role of early and then chronic epidermal barrier dysfunction in the pathophysiology of this evolution.^{6–8}

Under normal conditions, the skin represents the organism’s main interface with the environment, normally protecting it against various external agents. In

patients with AD, however, ceramides depletion, filaggrin (FLG) deficiency, and/or other cutaneous defects produce epidermal barrier impairment, with greater skin permeability in both an inside-outside and an outside-inside direction.⁹ These alterations allow irritants and allergens to penetrate the skin and trigger inflammatory responses, which could be preferably T-helper (Th) 2 driven because of the deviation of the immune system that is characteristic of individuals with atopy. The subsequent allergic sensitization results in the production of specific immunoglobulin E (IgE) and memory T cells. The shift toward allergic respiratory manifestations could then be explained by the systemic recirculation of such elements.^{10,11} This hypothesis has been confirmed by murine models: prolonged and repeated epicutaneous application of ovalbumin in mice induced local skin inflammation predominantly of the Th2 type, not only increasing the levels of specific IgE against the protein but also triggering hyperresponsiveness to methacholine after inhalatory exposure to the same allergen.^{12,13}

A complete clinical assessment of patients with AD should include an evaluation of the degree of skin barrier impairment. The most useful tools for this purpose to date are the Tewameter (Tewameter TM300;

From the ¹Section of Pediatrics, Department of Life and Reproduction Sciences, University of Verona, Verona, Italy, and ²Section of Pediatrics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

The authors have no conflicts of interest to declare pertaining to this article

Address correspondence to Diego G. Peroni, M.D., Pediatric Clinic, University of Ferrara, Via A. Moro, 8, Ferrara, Italy

E-mail address: diego.peroni@unife.it

Copyright © 2015, OceanSide Publications, Inc., U.S.A.

CK Electronic GmbH, Cologne, Germany) and the Corneometer (Corneometer CM825; CK Electronic). The former measures transepidermal water loss (TEWL), defined as the amount of water that passes from inside the body through the epidermal layer to the surrounding atmosphere *via* diffusion and evaporation processes; and the latter evaluates stratum corneum hydration. These biophysical parameters have been extensively validated as a means of instrumental evaluation for skin barrier function in AD.^{14,15} TEWL and Corneometer evaluations, as accurate markers of epidermal barrier impairment, can be effectively used to point out the importance of epicutaneous sensitization to aeroallergens in AD. There are studies in the literature that report the use of these measurements for this purpose, both in animal models¹⁶ and in infants.¹⁷

However, taking into account a broader age range could be helpful because increasing age means a longer time of exposure of the atopic skin to environmental agents, which leads to a greater potential influence on the development of allergic sensitization. Moreover, all the studies restricted research to examining the correlation between epidermal barrier impairment and the presence of aeroallergen sensitization, without evaluating its severity and extent. The aim of our study was to investigate this perspective in an all-ages pediatric population by assessing AD skin by means of TEWL and Corneometer evaluations, and investigating for a correlation between barrier integrity and the level of aeroallergen sensitization, as expressed by the severity of wheal reaction to skin-prick testing.

METHODS

Patients

Our study enrolled children with a diagnosis of AD according to the U.K. Working Party's diagnostic criteria.¹⁸ The children were seen consecutively in the Allergy Outpatient Clinic at the Department of Pediatrics of the University of Verona, Italy. Patients were recruited between October 2013 and April 2014. A non-AD group was simultaneously enrolled, which included children with allergy and those without allergy seen in the same clinic. The only exclusion criteria was a positive personal history of cutaneous disorders. The local ethics committee approved the study. The parents of the children were adequately informed about the methods and aims of the study and freely decided on conscious involvement in the research after giving their written informed consent.

Clinical Assessment

The children with AD were given a thorough medical examination to evaluate the percentage of skin involvement and the severity of eczematous lesions. The severity of AD was evaluated by means of the Scoring

Atopic Dermatitis (SCORAD) index, which combines objective parameters of extent and intensity of clinical signs (*i.e.*, erythema, edema and/or papulation, excoriations, lichenification, oozing and/or crusts, and dryness) with subjective criteria (pruritus and sleep loss).¹⁹ According to the SCORAD index, eczema was scored in each patient as mild (≤ 35), moderate (35–60) or severe (> 60), as defined in previous studies.²⁰ Scores were determined by using specific personal computer software (ScoradCard; TPS Production, Rome, Italy).

Dermatologic Assessment

TEWL measurements were taken by using a Tewameter (Tewameter TM300; CK electronic GmbH, Cologne, Germany) on every lesion site observed at the physical examination; an area of healthy skin, localized in most cases on the extensor surface of the upper arm, was likewise evaluated in each patient. For an adequate reading, children had to remain perfectly still during the entire procedure, and the Tewameter probe was held in place for the time needed to obtain a steady value in accordance with published guidelines.²¹ Stratum corneum hydration was measured by means of a Corneometer on the same areas as for the TEWL assessment. To ensure reproducibility of measurements, all the assessments were performed in the same room, with standardized temperature and relative humidity conditions, by the same trained physician (F.D.M.).²¹

Allergological Evaluation

Skin-prick tests were performed on uninvolved skin at the volar surface of the forearm with standardized allergen extracts (Stallergenes, Milan, Italy) for 21 inhalant allergens, including grass (grass mixture and dog's tooth grass), herbs (ambrosia, ribwort plantain, nettle, wormwood, *Parietaria*), trees (beech, olive tree, tree mixture, cypress, mimosa), epidermals (horse, feather mixture, cat, dog), mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), and fungi (*Alternaria*, *Aspergillus*, and *Cladosporium*). Histamine phosphate (10 mg/mL) and physiologic saline solution were used as positive and negative controls, respectively. The cutaneous response was scored 15 minutes after challenge as the greatest wheal diameter expressed in millimeters.²² Global severity of allergic sensitization was assessed for each patient by summing all of the single wheal diameters, excluding histamine; the quantitative score obtained was subsequently referred to as the allergy score (the quantitative score that estimates the severity of a patient's allergic sensitization, derived from the sum of all the single wheal diameters obtained at skin-prick testing).²³

Statistical Analysis

Results are expressed as mean (standard deviation [SD]); differences were considered significant at $p <$

0.05. A comparison of TEWL and Corneometer measurements between lesion site and healthy skin and between healthy skin of subjects with AD and subjects without AD was performed by using the rank sum test. Among groups that presented different AD severities according to the SCORAD index, a comparison of TEWL and Corneometer evaluations was done by using the Kruskal-Wallis rank test. The linear regression method was used to study the association between the allergy score and disease duration and healthy skin's TEWL.

The required sample size has been estimated *a priori* with a *t*-test for the difference of mean TEWL of non-lesional skin in two independent populations (children with and children without AD), according to the following assumptions: a significance level of $\alpha = 5\%$, a power of $\beta = 80\%$, an allocation ratio $n_2/n_1 = 3$, and an expected standard deviation of $7 \text{ g/m}^2/\text{hr}$.²⁴ The expected difference between means was $5 \text{ g/m}^2/\text{hr}$, which corresponds to an expected effect size ~ 0.7 . Under these assumptions, the estimated sample size was equal to 21 subjects without AD and 63 subjects with AD. All statistical analyses were performed by using GraphPad Prism for Windows (GraphPad Software, La Jolla, CA).

RESULTS

Sixty-one children affected by AD, ages between 6 months and 17 years, were enrolled in the study. According to the SCORAD index, AD was mild in 28 subjects (45.9%), moderate in 26 (42.6%), and severe in 7 (11.5%). The non-AD group consisted of 20 subjects, ages 3–15 years. The general characteristics of the population studied are shown in Table 1.

TEWL and Corneometer levels were globally measured in the patients with AD on 12 different lesion sites; to avoid bias caused by the diverse locations, however, statistical analysis was done by considering only those measurements on antecubital fossa lesions. Values referred to healthy skin derived for the same reason from a unique area located on the extensor surface of the upper arm for both AD and non-AD groups. Data are summarized in Table 2. As expected in patients with AD, TEWL and Corneometer measurements on lesion sites showed important differences compared with measurements on healthy skin of the same patient, with TEWL significantly higher ($p < 0.0001$) and skin hydration significantly lower ($p = 0.011$) on visibly damaged areas (data not shown). Moreover, when we compared the values of TEWL and Corneometer results between the two study groups, it emerged that children with AD had a significantly greater TEWL, a sign of barrier defect, on healthy skin compared with subjects without AD ($p = 0.017$) (Fig. 1). The same comparison in relation to the values of stratum corneum hydration did not give significant results.

Table 1 Demographic and allergic characteristics of patients included in the study

	AD (n = 61)	Non-AD (n = 20)
Mean (SD) age, y	5.92 ± 4.23	8.8 ± 3.5
Boys, no. (%)	33 (54.1)	15 (75)
Girls, no. (%)	28 (45.9)	5 (25)
Parental history of atopy, no. (%)	27 (44.3)	8 (40)
Allergic sensitization, no. (%)		
Inhalant allergy	36 (59)	14 (70)
None	25 (41)	6 (30)
Respiratory symptoms, no. (%)		
Asthma	15 (24.6)	15 (75)
Rhinitis	8 (13.1)	7 (35)
None	40 (65.6)	3 (15)
Severity of AD, no. (%)		
Mild (SCORAD ≤ 35)	28 (45.9)	–
Moderate (SCORAD = 35–60)	26 (42.6)	–
Severe (SCORAD > 60)	7 (11.5)	–

Table 2 TEWL and Corneometer values (mean [SD])

Area	TEWL (g/m ² /hr)	Corneometer (u)
AD		
Face lesion	32.33 ± 17.15	30.16 ± 16.46
Neck lesion	41.87 ± 18.35	36.27 ± 19.85
Axilla lesion	28.15 ± 21.99	26.75 ± 5.73
Upper arm lesion	20.38 ± 13.83	24.92 ± 11.59
Elbow lesion	26.82 ± 11.58	31.17 ± 13.59
Forearm lesion	20.83 ± 16.61	21.3 ± 5.72
Hand lesion	28.67 ± 15.32	27.33 ± 15.09
Abdomen lesion	25.38 ± 23.36	35.03 ± 13.21
Back lesion	27.7 ± 17.54	25.39 ± 7.84
Groin lesion	25.06 ± 24.92	42.62 ± 32.18
Knee lesion	32.5 ± 16.63	34.2 ± 18.33
Foot lesion	35.53 ± 23.29	50 ± 26.12
Healthy skin	11.72 ± 7.51	36.65 ± 12.37
Non-AD		
Healthy skin	8.96 ± 6.49	38.78 ± 12.72

When evaluating the subjects according to their SCORAD index and TEWL and Corneometer values, alterations were confirmed and showed in addition a significantly direct relationship with severity of disease. In particular, the more severe the disease, the

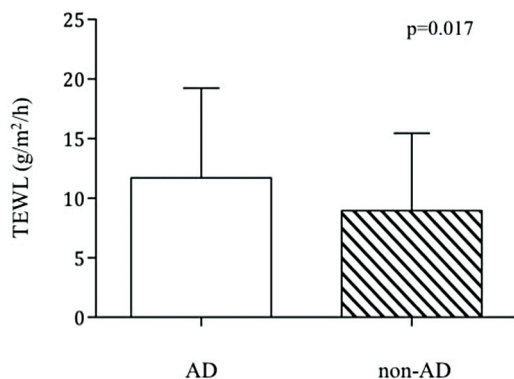


Figure 1. TEWL of healthy skin in subjects with AD versus subjects without AD.

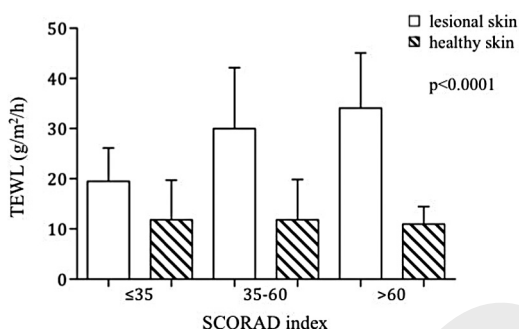


Figure 2. TEWL readings according to the SCORAD index.

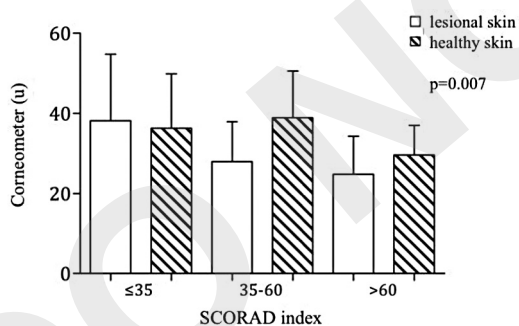


Figure 3. Corneometer readings according to the SCORAD index.

greater the TEWL on lesional skin (mean [SD] 19.48 ± 6.62 g/m²/hr in patients with mild AD versus mean [SD] 29.96 ± 12.16 g/m²/hr in patients with moderate AD versus mean [SD] 34.04 ± 11.02 g/m²/hr in patients with severe AD; $p < .0001$) (Fig. 2). Conversely, Corneometer values on eczematous areas decreased (38.17 ± 16.60 corneometer units in subjects with mild disease versus 27.97 ± 9.94 corneometer units in subjects with moderate disease versus 24.83 ± 9.50 corneometer units in subjects with severe disease; $p = 0.007$) (Fig. 3). Atopic familial history was globally well represented in both groups (44.3% in children with AD and 40% in children without AD). Interestingly, parental history of AD was documented only for seven children in the AD group.

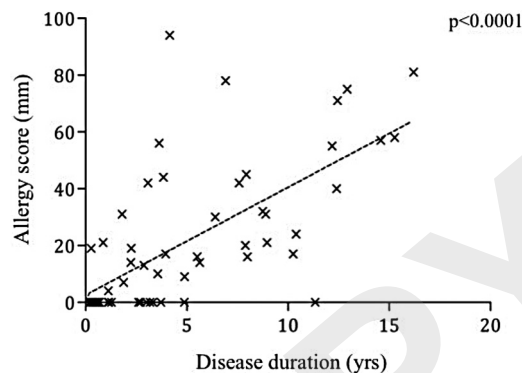


Figure 4. Correlation between AD duration and allergy score.

The prevalence of allergic sensitization in the samples was generally high (Table 1). In the AD group, only 25 of 61 children (41%) did not show any positive results to skin-prick tests, with 59% of individuals showing sensitization to aeroallergens; among individuals with allergy, most (33 of 36) were polysensitized, with documented reactions to at least two inhalant allergens. Allergy scores showed a mean (SD) value of 34.94 ± 24.01 mm. Respiratory symptoms (allergic rhinitis and/or asthma) were present in 21 of 61 children with AD. In the non-AD group, 14 of 20 children (70%) proved to be allergic, with only one child who was monosensitized. Allergy scores showed a mean (SD) value of 26.14 ± 11.76 mm. Seventeen of 20 children showed respiratory symptoms (allergic rhinitis and/or asthma). The prevalence of aeroallergen sensitization was substantially similar in the two study groups (bearing in mind that the children without AD were nevertheless subjects with atopic diathesis).

When considering individuals with allergy in both research groups, all possible correlations between allergy score and skin parameters in the study were then analyzed to identify any significant associations between the degree of sensitization to inhalant allergens and epidermal barrier conditions. In patients with allergic AD, a significant positive correlation was found between the allergy score and disease duration ($r = 0.63$; $p < 0.0001$) (Fig. 4): children affected by the disease for a longer time showed significantly greater aeroallergen sensitizations. Remarkably, in the same patients, allergy scores positively correlated with the TEWL value of nonlesional skin ($r = 0.46$; $p = 0.002$) (Fig. 5): higher TEWL levels on apparently healthy sites, suggestive of the baseline conditions of the patient's skin, were associated with the greater allergy scores and, therefore, with the greater allergic sensitizations to inhalants. The search for a correlation between Corneometer values and allergy scores did not show any positive results. In the non-AD group, no significant correlation was found between the allergy score and skin parameters.

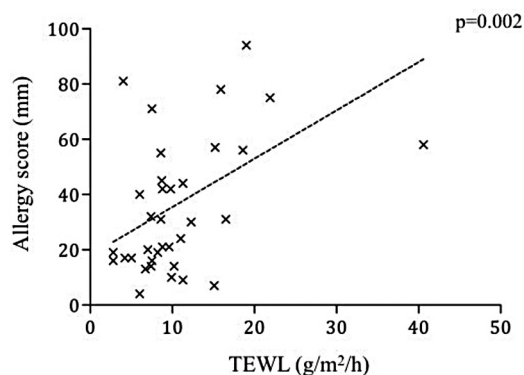


Figure 5. Correlation between TEWL of nonlesional skin of patients with allergic AD and allergy score.

DISCUSSION

AD is a chronic relapsing inflammatory skin disease whose main cornerstone is represented by epidermal barrier impairment. Important instruments for investigating this defect are TEWL and Corneometer, which are able to detect the condition of skin barrier function. We used these tools in our study to objectively define the skin condition of patients with AD, in search of an association between barrier integrity and degree of sensitization to aeroallergens. We eventually reached the conclusion that, in children with AD, higher TEWL readings of healthy skin (suggestive of the individual's basal skin conditions) corresponded to greater severity of allergic sensitization; in other words, a significant barrier defect allows excessive skin permeability, which leads to more probable inhalant allergen sensitization. Moreover, a positive association was observed between the allergy score and disease duration, which implies that a prolonged time of exposure to environmental allergens of a skin bearing an intrinsic defect of barrier function runs a higher risk of allergic sensitization. When considering that subjects with atopy are *per se* predisposed to the development of allergic manifestations, the presence of allergic sensitization to aeroallergens constitutes, in this kind of subject, an additional risk factor for atopic progression.²⁵ Indeed, of the 36 children with allergic AD enrolled in the study, 20 (56%) already presented with allergic rhinitis and/or with asthma at the time of recruitment.

The results of the present study concord with other studies in this field. In fact, clinically damaged skin areas in patients with AD seemed to have, as expected,⁹ significantly higher TEWL and significantly lower skin hydration compared with healthy skin of the same subject. Moreover, increasing severity of the disease was associated with proportional variations of these parameters: slightly higher TEWL and lower Corneometer values invariably reflected progressively greater skin barrier impairment and were thus associated with more severe clinical manifestations of AD, as

already described.²⁶ Furthermore, we showed that uninvolved skin of children with AD differed from that of children without AD by showing significantly higher TEWL values. In fact, as previously observed by different research groups, nonlesional skin of patients with AD proves to be altered compared with the skin of subjects without AD and, therefore, should be referred to as "seemingly healthy skin."^{26,27} It has also recently been shown that the stratum corneum of lesional but also of clinically unaffected skin of adults with AD is abnormal, with reduced expression of FLG and FLG-like proteins.²⁸

The connection between AD and allergic sensitization has been studied by many clinicians over the past decades, but it remains unclear whether allergic sensitization should be considered a cause or a consequence of eczema. To date, some investigators have indicated that both options could be possible²⁹; nevertheless, there is a growing body of evidence that eczema can induce allergic sensitization, especially as concerns food allergens.³⁰ With regard to aeroallergens, our study showed that epidermal barrier impairment in patients with AD can also be a strong risk factor for the development of sensitization. Individuals affected by the disease bear an intrinsic generalized skin barrier defect in which several genetic and environmental factors intervene to trigger the inflammatory responses responsible for eczema re-exacerbations.¹⁶ Causes of this basal alteration of the skin could be found in mutations of various genes coding for different stratum corneum components, *e.g.*, FLG³¹ or serine-proteases inhibitor Lympho-epithelial Kazal-type-related inhibitor,³² as already indicated by numerous genetic studies. In addition to genetic modifications of FLG and FLG-like proteins, proinflammatory cytokines and defects in pro-FLG processing can cause FLG down-regulation, which indicates that skin inflammation, in turn, may reduce the expression of FLG and FLG-like proteins, which contribute to the AD-related epidermal barrier dysfunction.²⁸ This barrier defect determines an increase in skin permeability in both directions, from inside the organism to the environment, with loss of water and electrolytes, and from the environment to inside the organism, with entrance of various types of substances, including irritants and aeroallergens.⁹⁻¹¹ These latter can thereby come into contact with the subject's immune system, which triggers immune responses, mainly of the Th2-type.

Many studies investigated the reasons for the activation of such responses that led to allergic sensitization. Results of some studies indicated the influence of tissue-specific factors that would generally draw epithelial cells to initiate Th2-type rather than Th1-type responses⁸; other studies, however, maintain that this T-cell pattern could be promoted only in individuals with associated predisposing "enhancer" alleles that

favor IgE responsiveness.¹⁰ Regardless of their exact way of production, Th2-specific lymphocytes remain in the skin-homing compartment as memory T cells, ready to be reactivated if the allergen could reach them once more. The loss of their homing-site-specific receptors and their subsequent systemic recirculation would then explain the switch of allergic manifestations from the cutaneous to the respiratory type and the progression from AD to allergic rhinitis and/or asthma.^{10,11}

The association between the skin barrier defect and the presence of sensitization to inhalant allergens has already been confirmed by studies in murine models and in children <1 year of age.^{16,17} Our study investigated the matter in a more-specific way, which revealed a significant correlation between epidermal barrier impairment and, not only the presence but also the level of sensitization to inhalant allergens. For this purpose, TEWL was used as an indicator of the outside-inside passage through the skin: although this parameter could be considered mainly as a marker for the inside-outside barrier, the majority of studies that investigated TEWL and percutaneous absorption found a quantitative correlation, which led to also considering TEWL as a marker for the outside-inside barrier.³³ The degree of sensitization to aeroallergens was expressed by the allergy score obtained for each patient by summing all of his or her single wheal diameters. This parameter has frequently been used in the literature to estimate the severity of allergic sensitization in patients^{34–36} and has been validated by Frith *et al.*,²³ who showed that the quantitative score derived from the sum of wheal diameters directly correlates with total serum IgE, which gives a good approximation of sensitization levels. It must be stressed that higher allergy scores imply not only greater individual sensitization to single allergens but also greater risks of polysensitization.³⁷ This undoubtedly constitutes an aggravating factor for the occurrence and severity of allergic diseases.³⁸

If these points can be claimed as strengths of our study, we must admit that our research had limitations. First of all, patients with both recent and dated diagnoses of AD were recruited, and compliance with possible ongoing treatments was not checked. Second, the analysis was limited to recording measurements in a single moment and did not evaluate the situation's evolution for each patient in time. Further research is necessary on larger cohorts of patients to confirm the association in single patients and long-term follow-up.

In summary, the present study provided evidence that the skin barrier was critical in protecting the organism against normally harmless environmental molecules that could otherwise trigger, in an individual with atopy, the activation of inappropriate defense systems. A defective skin barrier significantly facilitated an encounter between allergens and one's im-

mune system, which led predisposed individuals to allergic sensitization and then most likely to the development of allergic diseases. It, therefore, is evident that restoration of skin barrier integrity needs to be a key factor in AD treatment. Adequate early topical therapy aimed to restore the stratum corneum not only on clinically damaged areas but also on seemingly healthy skin could be of utmost importance in controlling the disease³⁹ and, accordingly, in preventing allergic sensitization and, in the long term, also progression toward allergic respiratory diseases.⁴⁰

ACKNOWLEDGMENTS

We thank Judyth Dillon for editing the English version of the manuscript.

REFERENCES

1. Darsow U, Wollenberg A, Simon D, et al. ETFAD/EADV Eczema task force 2009 position paper on diagnosis and treatment of atopic dermatitis. *J Eur Acad Dermatol Venereol* 24:317–328, 2010.
2. Illi S, von Mutius E, Lau S, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 113:925–931, 2004.
3. Gustafsson D, Sjöberg O, and Foucard T. Development of allergies and asthma in infants and young children with atopic dermatitis — a prospective follow-up to 7 years of age. *Allergy* 55:240–245, 2000.
4. Spergel JM, and Paller AS. Atopic dermatitis and the atopic march [review]. *J Allergy Clin Immunol* 112(suppl.):S118–S127, 2003.
5. Leonardi S, Cuppari C, Manti S, et al. Serum interleukin 17, interleukin 23, and interleukin 10 values in children with atopic eczema/dermatitis syndrome (AEDS): Association with clinical severity and phenotype. *Allergy Asthma Proc* 36:74–81, 2015.
6. Dohi M, Okudaira H, Sugiyama H, et al. Bronchial responsiveness to mite allergen in atopic dermatitis without asthma. *Int Arch Allergy Immunol* 92:138–142, 1990.
7. Tinkle SS, Antonini JM, Rich BA, et al. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ Health Perspect* 111:1202–1208, 2003.
8. Cookson W. The immunogenetics of asthma and eczema: A new focus on the epithelium. *Nat Rev Immunol* 4:978–988, 2004.
9. Proksch E, Brandner JM, and Jensen JM. The skin: An indispensable barrier. *Exp Dermatol* 17:1063–1072, 2008.
10. Taïeb A. Hypothesis: From epidermal barrier dysfunction to atopic disorders. *Contact Dermatitis* 41:177–180, 1999.
11. Li M. Current evidence of epidermal barrier dysfunction and thymic stromal lymphopoietin in the atopic march. *Eur Respir Rev* 23:292–298, 2014.
12. Spergel JM, Mizoguchi E, Brewer JP, et al. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 101:1614–1622, 1998.
13. Leyva-Castillo JM, Hener P, Jiang H, and Li M. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. *J Invest Dermatol* 133:154–163, 2013.
14. Holm EA, Wulf HC, Thomassen L, and Jemec GB. Instrumental assessment of atopic eczema: Validation of transepidermal wa-

- ter loss, stratum corneum hydration, erythema, scaling, and edema. *J Am Acad Dermatol* 55:772–780, 2006.
15. Addor FAS, Takaoka R, Rivitti EA, and Aoki V. Atopic dermatitis: correlation between non-damaged skin barrier function and disease activity. *Int J Dermatol* 51:672–676, 2012.
 16. Kondo H, Ichikawa Y, and Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur J Immunol* 28:769–779, 1998.
 17. Boralevi F, Hubiche T, Léauté-Labrèze C, et al. Epicutaneous aeroallergen sensitization in atopic dermatitis infants — Determining the role of epidermal barrier impairment. *Allergy* 63: 205–210, 2008.
 18. Williams HC, Burney PGJ, Pembroke RJ, et al. The U.K. Working Party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol* 131:383–416, 1994.
 19. Severity Scoring of Atopic Dermatitis: The SCORAD Index. Consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 186:23–31, 1993.
 20. Kunz B, Oranje AP, Labrèze L, et al. Clinical validation and guidelines for the SCORAD index: Consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 195: 10–19, 1997.
 21. Rogiers V, and EEMCO Group. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol Appl Skin Physiol* 14:117–128, 2001.
 22. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test — European standards. *Clin Transl Allergy* 3:3, 2013.
 23. Frith J, Fleming L, and Bossley C. The complexities of defining atopy in severe childhood asthma. *Clin Exp Allergy* 41:948–953, 2011.
 24. Kelleher M, Dunn-Galvin A, Hourihane JOB, et al. Skin barrier dysfunction measured by transepidermal water loss at 2 days and 2 months predates and predicts atopic dermatitis at 1 year. *J Allergy Clin Immunol* 135:930–935.e1, 2015.
 25. Spergel JM. Epidemiology of atopic dermatitis and atopic march in children. *Immunol Allergy Clin North Am* 30:269–280, 2010.
 26. Polańska A, Dańczak-Pazdrowska A, Silny W, et al. Nonlesional skin in atopic dermatitis is seemingly healthy skin — Observations using noninvasive methods. *Wideochir Inne Tech Malo Inwazyjne* 8:192–199, 2013.
 27. Gupta J, Grube E, Ericksen MB, et al. Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. *J Allergy Clin Immunol* 121:725–730.e2, 2008.
 28. Pellerin L, Henry J, Hsu C, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 131:1094–1102, 2013.
 29. Lowe AJ, Abramson MJ, Hosking CS, et al. The temporal sequence of allergic sensitization and onset of infantile eczema. *Clin Exp Allergy* 37:536–542, 2007.
 30. Shibuya N, Saito E, and Karasawa C. Dermatitis in early infancy as a risk factor for sensitization and allergic diseases during the first year of life [in Japanese with English abstract]. *Arerugi* 62:1598–1610, 2013.
 31. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38:441–446, 2006.
 32. Fortugno P, Furio L, Teson M, et al. The 420K LEKTI variant alters LEKTI proteolytic activation and results in protease deregulation: Implications for atopic dermatitis. *Hum Mol Genet* 21:4187–4200, 2012.
 33. Levin J, and Maibach H. The correlation between transepidermal water loss and percutaneous absorption: An overview. *J Control Release* 103:291–299, 2005.
 34. Peroni DG, Piacentini GL, Cametti E, et al. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol* 164:1078–1082, 2011.
 35. Koh YI, and Choi S. Blood eosinophil counts for the prediction of the severity of exercise-induced bronchospasm in asthma. *Resp Med* 96:120–125, 2002.
 36. Sugnam KK, Collins JT, Smith PK, et al. Dichotomy of food and inhalant allergen sensitization in eosinophilic esophagitis. *Allergy* 62:1257–1260, 2007.
 37. Passalacqua G, Melioli G, Bonifazi F, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. *Allergy* 68:1029–1033, 2013.
 38. Burbach GJ, Heinzerling LM, Edenharter G, et al. GA(2)LEN skin test study II: Clinical relevance of inhalant allergen sensitizations in Europe. *Allergy* 64:1507–1515, 2009.
 39. Boguniewicz M. Atopic dermatitis: The updated practice parameter and beyond. *Allergy Asthma Proc* 35:429–434, 2014.
 40. Leung DYM, and Guttman-Yassky E. Deciphering the complexities of atopic dermatitis: Shifting paradigms in treatment approaches. *J Allergy Clin Immunol* 134:769–779, 2014. □