Skin barrier status during dupilumab treatment in patients with severe atopic dermatitis

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Abstract

Background: Atopic dermatitis (AD) is a common chronic-relapsing inflammatory skin disease hallmarked by epidermal barrier dysfunction, increased transepidermal water loss (TEWL) and decreased skin hydration. Recent findings on the T helper 2 (Th2)-driven pathogenesis of AD have led to the development of dupilumab, a monoclonal antibody directed against interleukin-4 and interleukin-13 that has been demonstrated to be effective in the treatment of moderate-to-severe AD. The effect of dupilumab on skin barrier dysfunction, however, has not yet been adequately investigated.

Objectives: The primary endpoint of this study was to assess the status of the skin barrier in nonlesional skin of patients with severe AD treated with dupilumab, by evaluating the association between the relative variation of TEWL and the achievement of a 75% reduction of EASI (EASI75) over time.

Methods: TEWL was measured below the antecubital fossae by means of the Vapometer® at baseline, at week 4 (T4), at week 16 (T16) and at week 32 after dupilumab starting. EASI and NRS-itch were measured at the same time points.

Results: Seventy-eight patients with severe AD treated with dupilumab were enrolled. Median TEWL relative variation respect to baseline was significantly higher in patients who achieved EASI75 as compared with those who did not achieve EASI75 at T16 and at T32, but not at T4.

Conclusion: During dupilumab treatment, TEWL on nonlesional skin tends to significantly improve 4 months after treatment initiation and could be a good tool for monitoring response to therapy.

Keywords: atopic dermatitis, dupilumab, skin barrier, transepidermal water loss, type 2 inflammation

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Introduction

Atopic dermatitis (AD) is a common, chronic-relapsing type 2–driven inflammatory skin disease clinically characterized by intense pruritus and recurrent eczematous lesions with a heterogeneous clinical presentation.[1]

Patients with moderate-to-severe AD often report a deterioration of their quality of life: severe pruritus leads to sleep disturbance, work productivity impairment, anxiety and depression. Although AD pathogenesis is complex and multifactorial, it is well established that two pivotal factors involved in its pathogenesis are (1) epidermal barrier disruption leading to an increase in transepidermal water loss (TEWL), a parameter related to the stratum corneum integrity,[2–5] and (2) immune dysregulation mainly consisting of T helper 2 (Th2) and T helper 22 (Th22) pathway upregulation resulting in overproduction of type 2

inflammation-related cytokines, such as interleukin (IL)-4, IL-13, IL-5, IL-31 and IL-22.[6,7] In particular, skin barrier abnormalities play a crucial role in the initiation and aggravation of AD and influence the immune dysfunction.[8] Genetic variants of filaggrin, a structural protein representing the major component of the keratohyalin granules involved in the keratinization, moisturization and antimicrobial properties of the human skin, are found in 15–40% of AD patients[9] and decreased levels of filaggrin and filaggrin-like proteins (hornerin and filaggrin family member 2) are found in lesional and nonlesional skin of AD patient who do not harbour the abovementioned genetic variants.[10,11] Ceramides, cholesterol and free fatty acids are the main constituents of the extracellular lipid matrix of the stratum corneum and are essential to ensure a correct barrier function. It has been described that ceramide ratio and ceramide/cholesterol ratio are reduced in AD skin[12,13] and hyperactivity of kallikrein along with increased levels of interferon α produce structural changes in free fatty acid and ceramide chains through an augmented degradation of very long chain fatty acid proteins (ELOV).[14] Elevated serine proteinase activity is also related to a reduction of the stratum corneum thickness and function due to the inactivation of acid sphingomyelinase and β-glucocerebrosidase, key enzymes in ceramide synthesis, and lamellar body secretion from keratinocytes, impairing keratinization process.[15] Tight junctions between keratinocytes of the granular layer act as a selective barrier, controlling cellular permeability; decreased levels of the transmembrane protein claudin-1, a major component of tight junctions, are strongly associated with AD leading to barrier function impairment and increased inflammation.[16] Although the AD standard treatment is based on emollients, topical and/or systemic corticosteroids, phototherapy and immunosuppressants [e.g. cyclosporine A (CsA)], recent findings on AD pathogenesis have led to the development of more targeted therapies. In particular, dupilumab is a monoclonal antibody that has been recently approved for the treatment of moderate-to-severe AD resistant to both topical agents and oral cyclosporine.[17–19] The proven clinical benefit of dupilumab on AD is regarded as depending on the dual blockade of the IL-4/IL-13 pathway. These cytokines are critical in the induction and perpetuation of the type 2 inflammation, but a possible ancillary effect of dupilumab on skin barrier integrity still needs to be investigated. Recent studies have shown that in patients treated with dupilumab, after 16 weeks of treatment the gene expression profile of lesional skin had shifted towards that of nonlesional skin, with an important reduction in the gene expression of the related Th2 cytokines. This phenomenon was accompanied not only by a reduction in the expression of the genes involved in the Th17/Th22 pathways but also by an upregulation in the expression of barrier components (e.g. filaggrin, loricrin, claudin and fatty acids) and significant reduction in epidermal hyperplasia.[20]

The primary objective of this prospective study was to assess the skin barrier status of nonlesional skin in patients with severe AD treated with dupilumab and investigate the association of TEWL improvement in nonlesional skin and achievement of 75% reduction of Eczema Area and Severity Index (EASI 75).

Methods

Patients
We performed a prospective study on 78 patients with the primary endpoint of assessing the skin barrier status in patients with severe AD treated with dupilumab. Inclusion criteria were (1) age $\geqslant$18 years, (2) severe disease defined by a baseline Eczema Area and Severity Index (EASI) $\geqslant$24 and (3) inadequate response/intolerance to CsA or medical inadvisability of CsA treatment. Patients with any documented psychiatric comorbidity were excluded from the study. All patients were given self-administered subcutaneous dupilumab 300 mg every other week following a loading dose of 600 mg. Traditional immunosuppressive agents (e.g. CsA, azathioprine and methotrexate) were discontinued at least 4 weeks before dupilumab initiation in all patients, while systemic corticosteroids were maintained in a minority of patients, with progressive tapering and subsequent withdrawal within 2 weeks. Concomitant topical corticosteroids or calcineurin inhibitors were allowed except for the antecubital fossa of the right arm, where TEWL was measured.

The full protocol was approved by the institutional review board of the ethics committee of the principal investigator's centre (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; protocol no. 696_2021). All the participants enrolled in the study gave

their written informed consent. The study was conducted between October 2020 and April 2021.

TEWL measurement and itch/disease severity assessment

TEWL (range = 0–300 g/m^2/h) was measured using Vapometer® (Delfin Technologies Ltd., Finland) by placing the instrument on a nonlesional area immediately below the antecubital fossa of the right arm, according to the standardized procedure described by Hon *et al.*[21]

Vapometer is a closed unventilated chamber system, validated by Nuutinen *et al.*[22] The reproducibility of Vapometer TEWL measures was assessed by Nuutinen *et al.*[22] and De Paepe *et al.*[23] A coefficient of variation equal to 8% was found in the initial work by Nuutinen *et al.*, relative to the very body area that we studied (volar forearm). TEWL measures are highly dependent on room microenvironment. Measurement conditions were standardized as follows: 40–50% relative humidity and 20°C room temperature. All measures were performed by a trained, dedicated dermatologist (SF). According to current literature, a 'normal' TEWL range does exist. Akdeniz *et al.*[24], however, reviewed the evidence on TEWL in healthy adults and found that the pooled TEWL estimate for proximal volar right forearm (corresponding to the area that we selected in this study) was 5.3 (4.3–6.3) g/m^2/h.

The final TEWL value was calculated averaging out the values obtained through three subsequent measurements. After this procedure, we evaluated the extension and severity of the disease by means of EASI. Itch was quantified through a Numeric Rating Scale (NRS) ranging from 0 to 10. TEWL, NRS-itch and EASI were collected at baseline (T0), week 4 (T4), week 16 (T16) and week 32 (T32) after dupilumab initiation.

Statistical analysis

Categorical variables were reported as frequencies and percentages while continuous variables were reported as medians and interquartile range (IQR). Relative variation of TEWL from baseline (T0) to T4, T16 and T32 was calculated. The nonparametric Wilcoxon two-sample test was used to assess if relative variation of TEWL, from t0 to the three time points considered, was different in patients who achieved 75% reduction of EASI (EASI75) from t0 to each time point, compared with those who did not achieve EASI75 at the same time point.

P values less than .05, two-sided, were considered statistically significant. The statistical software SAS (release 9.4, SAS Institute, Inc., Cary, NC, USA) was used to perform all the statistical analyses.

Results

Seventy-eight patients (29 males and 49 females) were enrolled. Clinical features of patients included in the study are summarized in Table 1. Disease onset in adult age (>18 years) was observed in 17 (21.79%) patients. Median age at dupilumab initiation was 35 (IQR = 24.75–46.5) years. At baseline, median EASI was 28 (IQR = 24–30.25), median NRS-itch was 8 (IQR =8–10) and median TEWL was 14 (IQR = 8–20) g/m^2/h (Figure 1). At baseline, only seven patients had a TEWL value within the normal range for the assessed area (<6.3 g/m^2/h).

EASI75 was achieved by 42 (53.85%), 57 (73.08%) and 63 (80.77%) patients at T4, T16 and T32, respectively. Median EASI reduction respect to baseline was of 21 (IQR = 18–25), 23 (IQR = 20–27) and 24 (IQR = 22–27) points at T4, T16 and T32, respectively. Median TEWL reduction respect to baseline was of 0 (IQR: –3.5 to –2), –3.7 (IQR: –8 to 0) and –5 (IQR: –11 to –0.6) points at T4, T16 and T32, respectively. Of the 71 patients with baseline TEWL values above the mentioned range (>6.3 g/m^2/h), 6 (8.5%), 9 (12.7%) and 11 (15.5%) patients achieved a normal TEWL at T4, T16 and T32, respectively.

A median 0% reduction of TEWL was documented in both the 53.85% patients achieving EASI75 at T4 and those who did not achieve EASI75 at the same timepoint.

A median 30% reduction of TEWL was documented in the 73.08% patients achieving EASI75 at T16, while only a 11% median reduction was noted in those who did not achieve EASI75 at the same timepoint (*p* = .0209).

A median 43% reduction of TEWL was documented in the 80.77% patients achieving EASI75 at T32, while only a 5% median reduction was

Table 1. Clinical features of patients with atopic dermatitis included in the study.

		Median (IQR)
Disease onset in adult age[a]		17 (21.79)
Age at dupilumab initiation (years)		35 (24.75–46.5)
Males[a]		18 (23.07)
Median clinical parameters at baseline	EASI	28 (24–30.25)
	NRS-itch	8 (8–10)
	TEWL	14 (8–20)
Median clinical parameters at T4	EASI	7 (3–11)
	NRS-itch	4 (3–5.25)
	TEWL	13.8 (9–19)
Median clinical parameters at T16	EASI	5 (2–7)
	NRS-itch	3 (2–5)
	TEWL	10 (7–14.25)
Median clinical parameters at T32	EASI	4 (1–6)
	NRS-itch	3 (1–5)
	TEWL	9 (7–12)

EASI, Eczema Area and Severity Index; IQR, interquartile range; NRS, Numeric Rating Scale; TEWL, transepidermal water loss.
[a]Data are reported as *n* (%).

noted in those that did not achieve EASI75 at the same timepoint ($p = .0088$) (Figure 2).

Discussion

Successful management of AD relies on restoring barrier function and decreasing local and systemic inflammation. In the daily practice, topical application of emollients is the cornerstone barrier repair therapy.[25] Ceramide-dominant emollients, which can help restore lipid barrier abnormalities, have been shown to improve TEWL values and upregulate antimicrobial peptides in the skin of atopic patients with similar efficacy compared with the topical calcineurin inhibitor tacrolimus.[26] Moreover, the reduction in TEWL has been demonstrated to parallel improvement of the severity scoring SCORAD (Scoring Atopic Dermatitis) together with the restoration of stratum corneum in a children population.[27] Other moisturizers containing α-hydroxyacid, pseudo-ceramides,

urea and hyaluronic acid have been demonstrated to be effective in ameliorating skin xerosis, itch and barrier permeability representing a safe and efficacy adjuvant therapeutic option to restore the barrier and sparring the use of topical corticosteroids, calcineurin inhibitors or both.[25] Currently, there are no data available investigating the role of systemic therapy on barrier function.

If, on the one hand, it is well acknowledged that skin barrier abnormalities, mainly resulting from decreased levels of filaggrin and ceramides, are major contributors in the pathogenesis of AD, on the other data on the status of skin barrier in patients with AD treated with dupilumab are limited. A study similar to ours has already been performed in a cohort of 30 AD patients treated with dupilumab, showing that there was an inverse proportional correlation between TEWL and disease severity after 8 weeks of dupilumab treatment.[28] Moreover, Rohner *et al.*[29] demonstrated in 10 AD

Figure 1. Clinical images showing improvement of atopic dermatitis in dupilumab-treated patients included in the study. (a–d) Face and neck: baseline, T4, T16 and T32. (e–h) Chest: baseline, T4, T16 and T32. (i–n) Popliteal fossa: baseline, T4, T16 and T32.
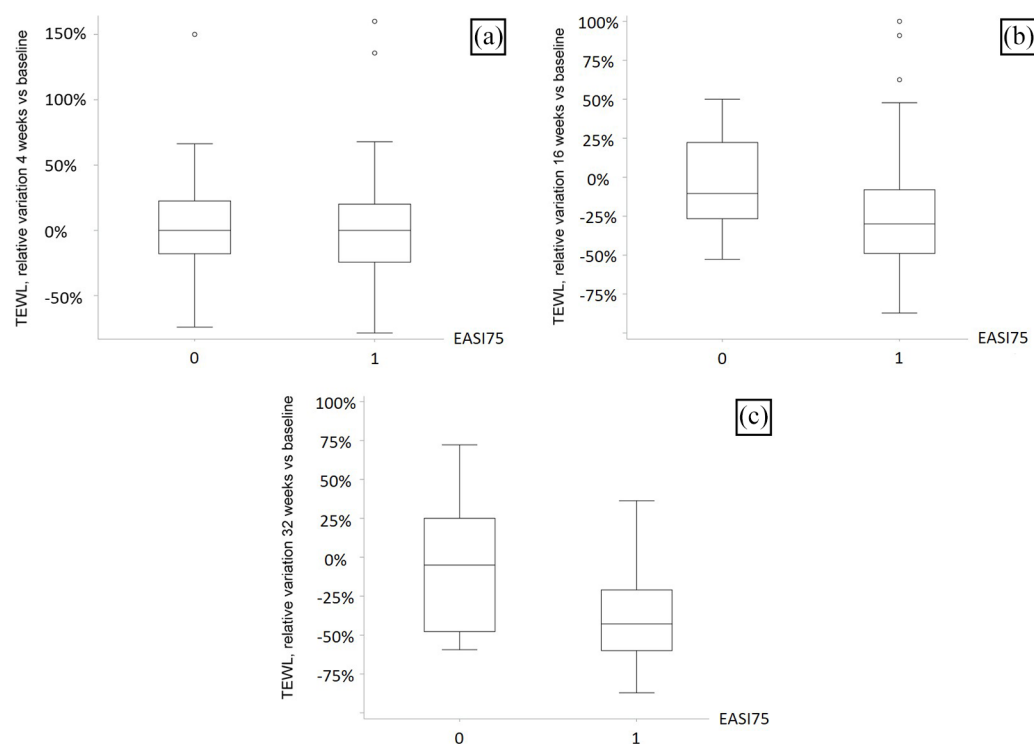
Figure 2. Box plots for TEWL relative variation in EASI75 achievers *versus* non-EASI75 achievers, at (a) 4, (b) 16 and (c) 32 weeks.

patients that dupilumab led to a significant increased expression of epithelial barrier proteins [filaggrin, lymphoepithelial-Kazal-type-related inhibitor (LEKTI), human β-defensin-3 and cathelicidin LL-37]. Similar conclusions were drawn by Guttman-Yassky *et al.*,[20] who found increased expression of the epidermal differentiation, barrier and lipid metabolism genes filaggrin, loricrin, claudins and ELOVL3. These results support that dupilumab therapy has the potential, over time, to change the history of this disease.

Our results confirmed the abovementioned findings and showed that dupilumab-induced EASI reduction is associated with improvement of skin barrier integrity measured through TEWL after the first 4 months from treatment initiation. It may be hypothesized that the reduction of skin inflammation related to dupilumab rapidly determines a reduction in EASI, while restoration of the barrier secondary to the anti-inflammatory action of dupilumab takes a longer period of time. More experimental evidence, however, is needed to unravel the mechanisms underlying skin barrier changes during dupilumab therapy.

The main limitation of this study is the small sample size, which was mainly due to its single-centre design. Selection bias due to the fact that the study included only patients with severe AD might be considered another limitation. Furthermore, even if we did our best to minimize any source of variation, emotional sweating, ambient relative humidity and skin surface temperature could have represented difficult-to-control environmental factors possibly influencing TEWL measurements.

Conclusion

In conclusion, our study demonstrates that TEWL, and thus skin barrier function in nonlesional skin, tends to significantly improve 4 months after treatment initiation paralleling disease severity amelioration measured by EASI.

Author contributions

SF, GG and AVM participated in generating original ideas and in study design. FG, ST, LA, GC and MR participated in conception and design or analysis and interpretation of data, or both. FG, ST and LA participated in revising the

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