

Letter to the Editor

***Staphylococcus aureus* density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis**

To the Editor:

Atopic dermatitis (AD) is a multifactorial skin disease with skin barrier damage, progressive sensitization to environmental antigens, and T_H2-skewed mediated systemic immune response.¹ Synthesis of filaggrin and filaggrin-like stratum corneum proteins is reduced both on lesional and on nonlesional skin of patients with AD irrespective of their *FLG* genotype.²

Staphylococcus aureus colonization of lesional skin in AD has been identified by traditional bacteriological sampling methods.³ We characterized, in comparison with healthy adults, a cohort of patients with AD in terms of AD phenotype, severity, *FLG* genotype, transepidermal water loss (TEWL), hydration index (HI), pH, pyrrolidone carboxylic acid (PCA) levels, and *S aureus* density on lesional and nonlesional skin. The objectives of the study were to (1) determine predictors of AD severity and (2) explore the abnormalities of nonlesional and lesional AD skin.

Adult patients with a diagnosis of AD defined by the Williams' criteria were selected. A cohort of nonatopic controls was recruited concomitantly from healthy volunteers from the Centre d'Etudes et de Recherches sur la Peau et les Epithéliums. Patients

and controls were matched for age and sex. All patients gave written informed consent. The study was approved by the Toulouse ethics committee.

AD disease course was evaluated with a standardized questionnaire. A European prick tests battery and measurement of total IgE in the serum were performed (ImmunoCAP 100, Pharmacia Diagnostics, Uppsala, Sweden). The severity of AD at presentation was determined by the Scoring Atopic Dermatitis (SCORAD) index.⁴ Severe AD was defined by a SCORAD index of more than 40. Disease course severity was estimated using the Nottingham Eczema Severity Score.⁵ TEWL, HI, and skin pH were measured with an Aquaflux camera (Biox Systems, London, United Kingdom), a Corneometer (CM825, Courage and Khasaka, Cologne, Germany), and a skin-pHmeter pH900C (Courage and Khasaka), respectively. For PCA measurement, LC-MS/MS analyses⁶ were carried out on an LCQ Deca XP Plus instrument interfaced with an electrospray ionization source (Thermo Electron, Courtaboeuf, France). Quantification of *S aureus* skin-estimated density by real-time PCR was assessed as previously described.⁷ For each parameter, measurements were performed on both lesional and nonlesional skin of patients with AD and on identical skin localizations of the matched controls. The 4 *FLG* mutations most prevalent in the European population (p.R501X, c.2282del4, p.S3247X, and p.R2447X) were investigated.²

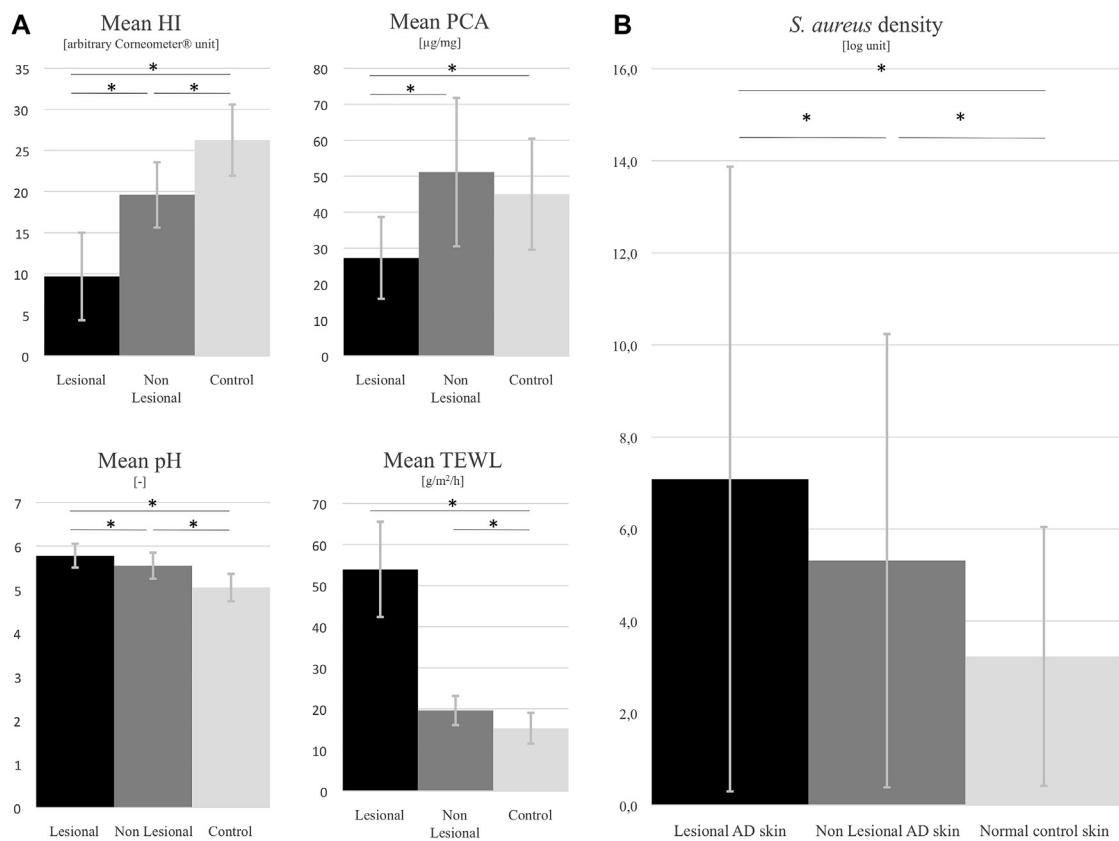


FIG 1. A, Investigation of skin barrier physicochemical properties in patients with AD (lesional and nonlesional skin) and controls. Error bars: SDs. * $P < .0001$. **B,** *Staphylococcus aureus* density in patients with AD (lesional and nonlesional skin) and controls. * $P < .0001$. Error bars: SEM.

TABLE I. Predictors of AD present and course severity measured by the SCORAD index and NESS: multivariable analysis

Score	Sample site	Effect	OR point estimates	95% CI
SCORAD index	Lesional skin	TEWL	2.53	0.77-8.25
		pH	1.36	0.43-4.27
		PCA	0.47	0.14-1.50
	Nonlesional skin	SPA	4.84	1.61-14.54
		TEWL	1.48	0.49-4.48
		IH	0.47	0.16-1.35
NESS	Lesional skin	SPA	4.34	1.51-12.47
		SPA*	1.79	0.42-7.57
	Nonlesional skin	SPA†	5.01	1.13-22.27
		pH*	1.40	0.30-6.60
		pH†	4.88	0.90-26.40
		SPA*	2.92	0.47-18.30
		SPA†	22.80	3.16-164.45

NESS, Nottingham Eczema Severity Score; SPA, Staphylococcus protein A.

*Mild group (reference) compared with moderate.

†Mild group (reference) compared with severe.

For statistical analysis (SAS version 8.2 Windows; SAS, Cary, NC), the following variables were included as potential predictive factors of AD severity in bivariable and multivariable logistic regression analyses: HI, pH, PCA, TEWL measurements, Staphylococcal skin density, and *FLG* gene mutations. For the analysis of severity, the SCORAD index was dichotomized between a value of less than 40 and a value of more than 40. The quantitative variables were dichotomized using the median value as cutoff. The variables displaying a *P* value inferior to .20 in the bivariable analysis were included in the multiple logistic regression analysis. A binary logistic regression analysis with stepwise selection of explanatory variables was performed as sensitivity analysis.

A total of 78 adult patients with AD were included (for patient's characteristics, see Table E1 in this article's Online Repository at www.jacionline.org). Eleven (14.1%) patients with AD exhibited 1 of the 4 most common mutations in the *FLG* gene compared with 4 controls (5.1%; *P* = .017).

Lesional and nonlesional skin of patients with AD showed lower HI and higher pH and TEWL compared with nonlesional skin and with healthy controls. PCA content was lower on lesional skin but similar on nonlesional and control skin (Fig 1). Staphylococcal density was significantly higher on lesional and nonlesional patient skin compared with control skin, with a 2.6 and 2.3 log ratio, respectively. It was higher on lesional than on nonlesional patient skin (Fig 1; see Table E2 in this article's Online Repository at www.jacionline.org).

A significant association was found between high staphylococcal density on lesional or nonlesional skin and severe AD (Table I). The sensitivity analysis was consistent with the primary analysis: considering lesional skin, the variables associated with severe AD as defined by a SCORAD index of more than 40 were *S aureus* density (odds ratio [OR], 5.4; 95% CI, 1.85-15.9) and TEWL (OR, 3.4; 95% CI, 1.17-10). When considering nonlesional skin, only *S aureus* density was significantly associated with severe AD (OR, 4.2; 95% CI, 1.6-11.3). A significant association was found between high staphylococcal density on lesional or nonlesional skin and AD course severity (*P* = .007 and .003, respectively). Ordinal logistic regression confirmed these results (Table I).

In AD, patients have increased bacterial colonization and infections with *S aureus*.³ Homeostasis between the host and the microbe is shifted during AD flares. Kong et al⁸ have revealed a dramatic change in microbial community structures in patients with AD compared to healthy volunteers and associated *S aureus* predominance in skin microbioma with flares of AD. Our study indicates a key association between *S aureus* density in both lesional and nonlesional skin and the present severity as well as the disease course severity of AD. Previous studies have shown that the presence of *S aureus* on lesional skin was more frequent in severe AD.³ Leung et al⁹ recently reported that the presence on lesional skin of *S aureus* producing enterotoxins and toxic shock syndrome toxin-1 correlated with clinical worsening when assessed by Investigator's Global Assessment, pruritus severity, and patient assessment score. Despite this increasing evidence, there is no consensus regarding therapeutic implications. Small studies have demonstrated that patients with AD can obtain benefit from the use of antibiotics in combination with corticosteroids and diluted bleach baths.⁸

Increased TEWL and reduced epidermal natural moisturizing factors are prevalent in clinically severe AD.¹⁰ Nonlesional skin of patients with AD displayed profound abnormalities in skin barrier and physicochemical properties as compared with control skin, irrespective of the presence of *FLG* null mutations. These results suggest that the concept of treating only lesional skin in patients with AD should be revisited. None of the skin barrier parameters was a predictor of AD instant severity or disease course.

S aureus density on both lesional and nonlesional skin appears to be the most important factor associated with AD severity using the SCORAD index and Nottingham Eczema Severity Score. It should be taken into account as a simple noninvasive prognosis tool and may lead to therapeutic applications.

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REFERENCES

1. Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483-94.
2. Pellerin L, Henry J, Hsu C-Y, Balica S, Jean-Decoster C, Méchin M-C, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 2013;131:1094-102.
3. Nilsson EJ, Henning CG, Magnusson J. Topical corticosteroids and *Staphylococcus aureus* in atopic dermatitis. *J Am Acad Dermatol* 1992;27:29-34.
4. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatol Basel Switz* 1993;186:23-31.
5. Emerson RM, Charman CR, Williams HC. The Nottingham Eczema Severity Score: preliminary refinement of the Rajka and Langeland grading. *Br J Dermatol* 2000;142:288-97.
6. Qu J, Chen W, Luo G, Wang Y, Xiao S, Ling Z, et al. Rapid determination of underivatized pyroglutamic acid, glutamic acid, glutamine and other relevant amino acids in fermentation media by LC-MS-MS. *Analyst* 2002;127:66-9.
7. Casas C, Ginisty H, Alvarez-Georges S, Viodé C, Lejeune O, Rouvrais C, et al. Molecular characterization of inflammation and *Staphylococcus aureus* colonization of involved skin of atopic dermatitis patients: a non-invasive approach. *Skin Pharmacol Physiol* 2008;21:260-8.
8. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012;22:850-9.
9. Leung DY, Hanifin JM, Pariser DM, Barber KA, Langley RG, Schlievert PM, et al. Effects of pimecrolimus cream 1% in the treatment of patients with atopic dermatitis who demonstrate a clinical insensitivity to topical corticosteroids: a randomized, multicentre vehicle-controlled trial. *Br J Dermatol* 2009;161:435-43.
10. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. *J Invest Dermatol* 2009;129:682-9.

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TABLE E1. Control and patient demographics and disease characteristics

Characteristic	Patients with AD (n = 78)	Controls (n = 78)
Age (y)	30 (18-49)	29.7 (18-49)
BMI (kg/m ²)	23.2 (16.2-36.3)	22.1 (18.4-30)
Personal history of atopy (%)	84.6	—
Smoker (%)	51.3	40.3
Standard prick test with at least 1 positive reaction (%)	98.7	79*
Elevated total IgE (>150 UI/mL) (%)	78.5	12.8*
SCORAD index	43.2 ± 14.34	—
NESS		
Mild	13/77	—
Moderate	29/77	—
Severe	35/77	—
DLQI	9.3 ± 5.04	—

BMI, Body mass index; *DLQI*, Dermatology Life Quality Index; *NESS*, Nottingham Eczema Severity Score.

**P* < .0001.

TABLE E2. *Staphylococcus aureus* density in patients with AD (lesional and nonlesional skin) and controls

Areas	Mean	Median	SD	Minimal value	Maximal value
Lesional AD skin	1.2×10^7	5.9×10^5	5.4×10^7	0.00	4.7×10^8
Nonlesional AD skin	2.1×10^5	2.2×10^4	7.4×10^5	0.00	5.0×10^6
Normal control skin	1.7×10^3	2.3×10^2	5.8×10^3	0.00	3.4×10^4

Units: number of gene copies/10 cm².

TABLE E3. Investigation of skin barrier physicochemical properties and functions in patients with AD (lesional and nonlesional skin) and controls

Areas	Studied parameter	Mean	Median	SD	Minimal value	Maximal value
Lesional AD skin	HI	9.68	7.32	10.68	−0.47	74.30
	pH	5.78	5.78	0.55	4.53	7.35
	TEWL	53.94	54.80	23.20	8.99	111.39
	PCA	27.25	21.89	22.71	1.15	123.68
Nonlesional AD skin	HI	19.60	18.54	7.95	6.23	45.37
	pH	5.55	5.70	0.60	4.31	6.57
	TEWL	19.60	18.15	7.08	9.59	42.33
	PCA	51.14	41.16	41.24	4.30	192.69
Normal control skin	HI	26.27	26.85	8.67	6.27	47.83
	pH	5.05	5.06	0.63	3.95	6.79
	TEWL	15.28	13.11	7.54	6.05	54.71
	PCA	45.02	41.24	30.87	9.81	190.25

HI (arbitrary Corneometer unit); PCA ($\mu\text{g}/\text{mg}$); TEWL ($\text{g}/\text{m}^2/\text{h}$).