Noninvasive measures in atopic dermatitis

Joachim W. Fluhr[a], *Torsten Zuberbier*[a], *and Razvigor Darlenski*[b,c]

Purpose of review
To summarize the current knowledge on the morphology, functionality and biochemical composition of the skin in allergic reactions. We address novel noninvasive techniques that promise to disclose intimate mechanisms of skin allergy *in vivo*. Epidermal barrier is not just a static wrap of the organism but rather a dynamic field for immunological, biophysical and biochemical processes and serves as a bio-sensor for exogenous danger signals.

Recent findings
Classical biophysical methods are amended by novel in-vivo techniques, such as Raman spectroscopy, analysing the skin microcomposition and develop epidermal profiles. Visualization techniques, such as reflectance spectroscopy and optical coherence tomography (OCT) are employed in studying the micro-morphological changes in the skin of allergic patients.

Summary
The noninvasive assessment of skin functions, micro-morphology and biochemical as well as immunological pathways will help to better understand skin allergies. They will allow to detect subtypes, for example in atopic dermatitis and to develop specific treatment modalities.

Keywords
atopic dermatitis, noninvasive assessment, Raman spectroscopy, stratum corneum hydration, transepidermal water loss

INTRODUCTION

Skin barrier with its most significant component, the stratum corneum, accomplishes competent protective function in healthy skin. Since its early introduction in the 1920s, the concept of the hydro-lipid acidic mantel of the skin has evolved [1]. Now we look at the stratum corneum as an active regulator of metabolic processes and functions. When the epidermal barrier is impeded, uncontrolled loss of water and electrolytes from the skin surface takes place facilitating the penetration of exogenous agents.

Atopic dermatitis is a model for impeded epidermal barrier function. It is a chronic inflammatory condition with high and increasing prevalence in the past decades [2]. Despite that the most prevalent outbreaks are in childhood, recent studies showed a high persistence in adulthood. [3]. The classic understanding of the disease pathophysiology involves Th2 immune dysregulation [4]. Since the discovery of the loss of function mutations in *filaggrin* genes in 2006 [5] the outside-in concept of atopic dermatitis pathogenesis has been introduced [6]. According to it, an epidermal barrier defect is requested to unlock to systemic inflammatory response in atopic dermatitis. Furthermore, impaired barrier-activated Th2 response predisposes to the development of food allergy and atopic march, that is asthma and allergic rhinitis [7,8].

Epidermal barrier disturbances with increased transepidermal water loss (TEWL) and decreased stratum corneum hydration have been demonstrated not only in the lesional skin of atopic dermatitis patients but also in clinically health uninvolved skin [9]. This corroborates the concept of persistent subclinical inflammation in clinically healthy atopic skin and a proactive therapy in atopic

[a]Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Department of Dermatology and Allergy, Comprehensive Allergy Center, Berlin Institute of Health, Berlin, Germany., [b]Department of Dermatology and Venereology, Acibadem City Clinic Tokuda Hospital, Sofia and [c]Department of Dermatology and Venereology, Trakia University, Stara Zagora, Bulgaria

Correspondence to Joachim W. Fluhr, Charite Universitätsmedizin Berlin, Department of Dermatology and Allergy, Charitéplatz 1, 10117 Berlin, Germany. E-mail: Joachim.fluhr@charite.de

Curr Opin Allergy Clin Immunol 2018, 18:000–000
DOI:10.1097/ACI.0000000000000476

1528-4050 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

KEY POINTS

- Allergy is a dynamic field and novel noninvasive techniques have been employed in studying skin allergic reactions
- The allergic skin possesses distinct morphological, functional and biochemical differences in comparison to healthy skin
- The most investigated morphological methods include OCT and reflectance spectroscopy
- Classical biophysical methods, such as assessment of transepidermal water loss, stratum corneum hydration and surface pH are employed in skin allergy
- Raman spectroscopy is a reliable in-vivo method for the detection of the biochemical composition in diseases

dermatitis [10]. In this review we address the classical noninvasive methods such as TEWL and stratum corneum hydration assessment together with novel techniques, for example in-vivo Raman confocal microspectroscopy (RCM), optical coherence tomography (OCT) and lipid analysis, assessing structural and functional abnormalities in atopic dermatitis. Figure 1 summarizes the morphological, functional, biochemical and genetic parameters that are altered atopic skin.

MORPHOLOGICAL CHANGES IN ATOPIC DERMATITIS

Reflectance spectroscopy

Reflectance spectroscopy is used in dermatology since approximately 10 years and has gained broader importance in noninvasive diagnostics of superficial skin cancer in the last 5 years. A customized reflectance probe for measurements in the near infrared region was used in efficacy testing of an emollient in eczema and atopic dermatitis. The authors showed increase in skin water content. The limitation of this study is the low number of volunteers and the lack of an adequate control group [11].

Gonzalez *et al* were one of the first to describe the features of irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) with reflectance spectroscopy [12,13]. They showed in 18 participants increased epidermal thickness mainly present in ICD, whereas ACD was characterized by microvesicle formation most prominent at 96 h following patch removal. Both ACD and ICD showed similar degree of exocytosis and spongiosis [12].

Sambacher *et al*. examined 12 patients with ACD using RCM, compared with classical H&E stained biopsies [14]. They analysed 22 lesions from 12 patch test patients. All ACD cases presented epidermal spongiosis, increased vascularization and inflammatory infiltrate. These features were most prominent in the stratum spinosum and granulosum. Depending on the severity of the patch test reaction, multiple microvesicles at the level of the

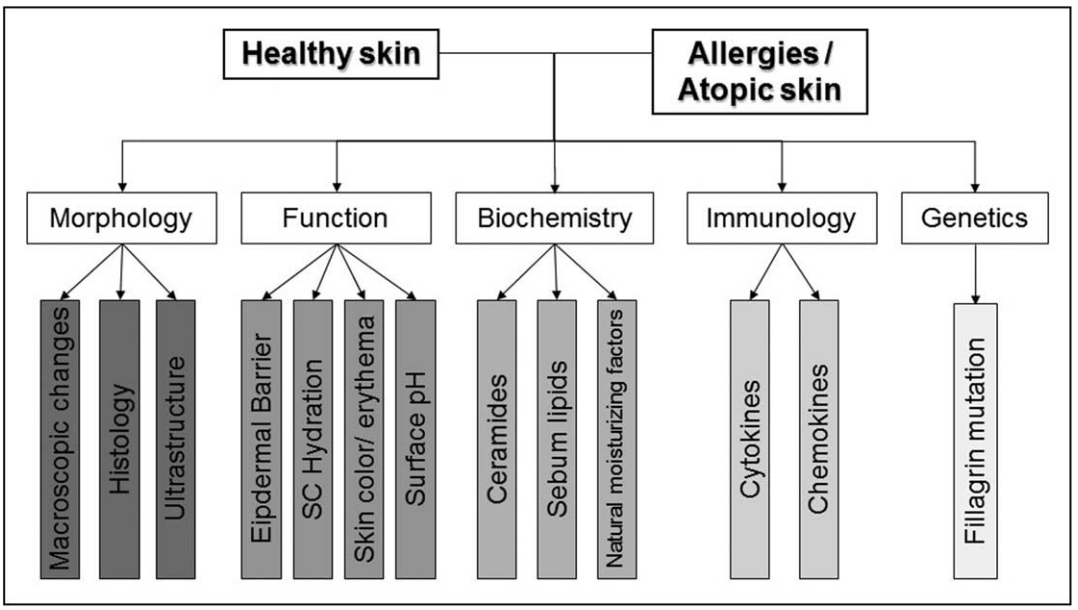

FIGURE 1. Summarizing the basis of a competent epidermis and their alteration in atopic dermatitis including morphology, function, biochemistry, immunology and genetics.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

stratum spinosum and granulosum were described in 17 of the 22 lesions. Densely packed, bright, roundish RCM structures at the stratum corneum level, corresponding to parakeratosis, were detected in ACD lesions. Furthermore, all cases with prominent parakeratosis showed the formation of microvesicles/vesicles.

Optical coherence tomography

One of the first studies using OCT was published by Gamblicher *et al.*: they were able to characterize ACD and correlate clinical grading of patch test reactions with OCT [15]. Twenty positive patch test reactions showed pronounced skin folds, thickened and/or disrupted entrance signals and a significant increase in epidermal thickness. Moreover, clearly demarcated signal-free cavities within the epidermis and considerable reduction of dermal reflectivity were demonstrated. The latter findings strongly correlate with the clinical patch test grading [15].

A multidisciplinary research group from Sheffield introduced angiographic OCT to assess subclinical atopic dermatitis severity in 32 atopic dermatitis patients with different local atopic dermatitis severities [16▪]. In atopic dermatitis patients without clinical symptoms, the superficial vascular plexus was deeper than in healthy patients. Superficial plexus depth correlated with the local severity of atopic dermatitis lesions. Additionally, severe atopic dermatitis showed larger vessel diameter, longer vessel segment length, higher vessel density and increased fractal dimension. These parameters provide semiquantitative metrics to assess subclinical severity of local atopic dermatitis severity, thus allowing monitoring of treatment effects past the end-point of clinical remission.

Aschoff *et al*. evaluated the atrophogenic potential of hydrocortisone 1% (HCT) and pimecrolimus 1% with OCT using high-frequency ultrasound as a comparative method [17]: epidermal thickness initially decreased in the HCT treated area already after 14 days. After 4 weeks, the epidermal thinning by HCT was higher (6.1%) than pimecrolimus (2.8%).

Boone *et al*. used a high-definition OCT (HD-OCT) a technique based on the principle of conventional OCT in 21 healthy volunteers and compared it to reflectance confocal microscopy [18]. They showed a 3-μm lateral resolution of the HD-OCT by phantom analysis. The identification of cells in the epidermis was achieved by both techniques. RCM offers the best lateral resolution, and HD-OCT has the best penetration depth, providing images of individual cells deeper within the dermis. Eccrine ducts and hair shafts can be visualized with pilosebaceous units depending on skin site. HD-OCT provides morphological imaging with sufficient resolution up to 570 μm in depth with the possibility for three-dimensional imaging [18].

The same group looked into differentiating OCT patterns of ACD versus ICD [19]. They were able to differentiate noninvasively the cytological and three-dimensional micro-architectural differences in skin reaction patterns between doubtful positive ACD and ICD in 22 patients with increased epidermal thickness observed in ICD. Vesicle formation and spongiosis was more prominent in ACD both in the stratum granulosum and the stratum spinosum especially in the reading after 96 h [19].

FUNCTIONAL CHANGES

Permeability barrier

Atopic skin shows impaired permeability function corresponding to an increased TEWL. Atopic dermatitis patients display increased TEWL even at clinically healthy skin when compared to healthy controls [9]. Others could not confirm the correlation between TEWL values and objective test for sensitive skin in atopic dermatitis [20]. Recently it was revealed that atopic dermatitis patients colonized with Staphylococcus aureus show greater skin barrier impediment, that is, higher TEWL values, than patient without colonization with the bacterium [21▪]. When assessing childhood eczema, a device-related difference was found between the correlation of clinical symptoms and TEWL [22]. Finally, elevated TEWL on the forehead of one-week-old babies was revealed as a predictor for atopic dermatitis development later in life regardless of their filaggrin mutation status [23].

Stratum corneum hydration

Atopic dermatitis patients have dryer skin than healthy person in both lesional and clinically healthy skin [9]. Capacitance measurement is a relevant method to discriminate stratum corneum hydration of lesional, perilesional and noninvolved skin [24]. A reason for that could be the systemic subclinical inflammation in uninvolved skin of atopic dermatitis patients as well as the impaired filaggrin degradation, which leads to insufficient components of the natural moisturizing factor (NMF) of the epidermis [25].

Assessment of stratum corneum hydration is widely used in cosmetic studies to prove efficacy of emollients, rinse-off products and topical formulations [26▪]. Stratum corneum hydration assessment has been employed in studying the effects of topical steroids versus calcineurin inhibitors in

1528-4050 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

atopic dermatitis [27]. It was shown that topical steroids but not calcineurin inhibitors interfere with the barrier properties and can decrease stratum corneum hydration [27]. The guidelines on measuring skin hydration have recently been updated [28].

Skin surface acidity

The 'acid mantle' of the skin is necessary to maintain and control bacteriological, chemical and mechanical resistance [29,30]. In addition to the traditionally accepted exogenous mechanisms of skin surface pH formation, three endogenous pathways have been proposed: generation of free fatty acids from phospholipids [31]; generation of *cis*-urocanic acid (UCA) by histidase-catalyzed degradation of histidine [32]; and via a sodium proton exchanger (NHE1) [33]. Skin acidification is essential for the epidermal barrier maturation and repair processes, for example regulation of enzyme activity exhibiting an acidic pH optimum [34]. Moreover, an increase of pH leads to degradation of these enzymes by inducing serine proteases activity [35,36]. Raising skin surface pH offers optimal conditions for the enzymatic action of kallikrein 5 (previously named stratum corneum trypsin-like enzyme) and kallikrein 7 (stratum corneum chymotrypsin-like enzyme) [37,38]. An elevation in stratum corneum pH resulted in reversible increase of the activity of these enzymes in a mammalian model [35]. It has been shown that atopic skin displays less acidic surface pH than healthy skin [39]. This difference is the basis for impaired skin barrier repair mechanisms in atopic dermatitis as well as a key factor in the increased desquamation [40]. The clinical observation of enhanced desquamation during the first days postpartum can be related to increased activity of the serine proteases in the more alkaline skin surface of atopic dermatitis patients. In addition, filaggrin mutations further contribute to the alkalization of the skin surface [41].

Acidification of stratum corneum prevents the progression from atopic dermatitis to respiratory allergy [42▪▪]. Decreasing the surface pH by acidic topical preparations has been shown effective in the enhancement of the skin barrier function in both humans and murine models of atopic dermatitis [43–45]. The clinical implication of this can be the development of more acidic topical formulations for atopic dermatitis [46].

Blood flow/microcirculation

Laser Doppler and laser speckle imaging can be used for blood flow mapping and imaging in the skin [47]. Skin blood flow reflects the level of inflammation. By means of two-photon microscopy the three-dimensional morphology of the skin blood vessels has been monitored in atopic dermatitis [48]. Thickened, flexuous blood vessels, associated with increased blood flow, in both erythematous and nonlesional areas were observed [48]. Neoangiogenesis and lymphangiogenesis have been linked to high levels of vascular endothelial growth factors-A and correlate with disease activity [49]. Circadian morning-to-evening discrepancies in the skin blood flow have been described in atopic dermatitis with a peak in the evening [50▪]. This influences the penetration of topically applied substances [51].

Sebum secretion

Decreased levels of sebum secretion have been witnessed in atopic dermatitis [52]. Different methods have been employed in the assessment of sebum lipids including sebumetry and the self-assessment tool – sebum check film [53]. A good correlation between the devices was evident showing decreased levels of sebum lipids in atopic dermatitis patients [53]. Deficient production of hexadecenoic acid, a sebum component, is associated with the vulnerability of atopic dermatitis patients to colonization by Staphylococcus aureus [54]. In addition, sebum components are important precursors of glycerol synthesis in the skin, a key player in the maintenance of skin hydration [55]. Hence, perturbed sebum secretion results in clinically dry skin in atopic dermatitis.

Eccrine sweating

The role of sweat in atopic dermatitis has been underestimated. By means of impression mold technique it was shown that sweating markedly decreased even in the earliest asymptomatic stage of atopic dermatitis, and the decrease was followed by compensatory hyperhidrosis at the ridge: leakage of sweat into the dermis could represent the initial event resulting in the decreased sweating and inflammation [56]. On the other hand, atopic dermatitis patients display hyperhidrosis and a dysfunction of sudomotor activity in the sympathetic nervous system witnessed by prolonged latency period of sympathetic skin response test [57▪,58]. Homologous proteins of MGL_1304 produced by Malassezia globose, identified as the major histamine releasing antigen in human sweat, have been revealed as potential sensitizers and can explain the sweat exacerbations of atopic dermatitis [59▪]. Sweat management for patients with atopic dermatitis is useful regardless of sweat allergy [60▪].

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

Biochemistry

Simpson *et al.* used several classical noninvasive instruments and Raman spectroscopy, to demonstrate an improvement of skin barrier function in 20 patients with controlled atopic dermatitis [61]. Topical moisturizer containing a ceramide precursor was applied twice per day on one leg versus the untreated opposite leg for 28 days. A reduced TEWL and clinical dryness scores, and increased skin hydration could be shown. Raman spectroscopy revealed a higher level of ceramide content at a depth of 5 and 10 μm [61]. The major drawback of this study is the lack of an adequate control of a cream not containing a ceramide precursor.

González *et al.* used Raman spectroscopy for the early detection of filaggrin-related atopic dermatitis [62]. They monitored 12 infants over one year. Three of them developed atopic dermatitis. The infants with a lower *FLG* content developed atopic dermatitis. This study should be confirmed with a higher number of infants as well as a genetic analysis for *FLG*-mutations.

The same group confirmed the 2282del4 and R501X mutations present in the European population in Mexican-mestizo patients with major complaints of pruritus and dry skin [63]. Eight out of the 19 patients with filaggrin gene mutations presented lower filaggrin concentrations using the vector correlation of their in-vivo skin Raman spectra.

Mischo *et al.* performed an ex-vivo analysis with Raman microspectroscopy in atopic dermatitis and HIV infection patients and age-matched healthy volunteers [64]. The analysis was performed on deeper skin layers, viable epidermis and dermis of 47 volunteers. A decrease in lipids within the viable epidermis for elderly and HIV-patients was detected. Decreases in lipids and simultaneous increases in water were demonstrated in the dermis for HIV and atopic dermatitis patients in comparison to healthy controls. This might be an explanation for altered stratum corneum observed in both diseases.

Mlitz *et al.* analysed the impact of *FLG* null mutations on biophysical properties and the molecular composition of the stratum corneum in 99 healthy individuals and 97 atopic dermatitis patients [65]. Stratum corneum concentrations of total NMF, water, ornithine and UCA were significantly lower in atopic dermatitis patients than in healthy controls. Null mutations of *FLG* were detected in 4% of controls and 10% of atopic dermatitis patients. *FLG* mutations were associated with increased stratum corneum levels of lactate, reduced concentrations of most other NMF components especially with higher disease severity in atopic dermatitis patients. In atopic dermatitis patients without *FLG* mutations, the content of NMF constituents decreased with increasing disease severity. The concomitant presence of low concentrations of NMF components in the stratum corneum was associated with *FLG* mutations with 92% specificity. This study is based on a large cohort using Raman Spectroscopy and genotyping for *FLG* mutations.

Janssen *et al.* studied in a case–control study the lipid/protein ratio and the total dry stratum corneum mass per surface area related to the skin barrier function of 28 patients with atopic dermatitis (14 carried at least one prevalent *FLG* null allele; 11 suffered from eczema lesions on at least one of the ventral forearms) and 15 controls [66]. They determined dry stratum corneum mass by tape-stripping and Squamescan. The ratio between lipid and protein bands in the Raman spectrum was used to determine the lipid/protein ratio. It could be shown that dry stratum corneum mass per skin area is altered only in lesional stratum corneum of patients with adverse event compared with control patients. The reduction in the lipid/protein ratio in stratum corneum of patients with adverse event was more pronounced, both in lesional and nonlesional stratum corneum and correlated with the skin barrier function and disease severity. The authors concluded that the lipid/protein ratio plays a more important role in the impaired skin barrier of adverse event than the stratum corneum thickness.

Specific Raman microspectroscopic NMF signatures of the stratum corneum could be used as markers of *FLG* genotype in patients with moderate-to-severe atopic dermatitis [67]. Three subpopulations with *FLG* genotype were identified by using Raman spectroscopy. The Raman signature of NMF discriminated between *FLG*-associated atopic dermatitis and non-*FLG*-associated atopic dermatitis. Within the subset of *FLG*-associated atopic dermatitis, NMF distinguished between patients with 1 versus 2 mutations. *FLG* mutations do not influence TEWL within established moderate-to-severe atopic dermatitis [67].

The same group investigated stratum corneum IL-1 cytokine profiles in the uninvolved skin in 137 patients with atopic dermatis with a *FLG* mutation versus patients with atopic dermatitis and no *FLG* mutation [68]. NMF levels were ascertained using confocal Raman spectroscopy; TEWL and skin surface pH were measured. IL-1α, IL-1β, IL-18, IL-1 receptor antagonist (IL-1RA) and IL-8 levels were determined in stratum corneum tape strips from 93 patients. All patients were screened for 9 *FLG* mutations. Stratum corneum IL-1 levels were increased in patients with atopic dermatitis (*FLG*); these levels were inversely correlated with NMF levels. NMF values were also inversely correlated

1528-4050 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

with skin surface pH. Atopic dermatitis (*FLG*) is associated with an increased stratum corneum IL-1 cytokine profile [68]. These findings support the hypothesis that *FLG* mutations have an influence on the inflammasome in the pathogenesis of atopic dermatitis.

Noninvasive assessment of barrier lipids

Sadowski *et al*. studied the epidermal lipid composition of human skin in quantitative high-throughput shotgun mass spectrometry-based platform from tape-stripped stratum corneum skin in 104 healthy participants from 14 anatomical sites [69▪]. Sixteen lipid classes; total quantification to the level of individual lipid molecules and relationship between sampling depth and lipid composition. They described sebaceous lipids to be a constitute of an abundant component of the stratum corneum lipidome as they diffuse into the topmost stratum corneum layers forming a gradient. Lipidomic variability with respect to sampling depth, site and participant were mainly accredited to sebaceous lipids, whereas stratum corneum lipids vary less [69▪]. Future studies should be performed regarding lipid profiles of dermatosis with a known defect of the epidermal barrier.

A recent paper form Jung *et al*. studied the effect of topical glucocorticosteroids, namely clobetasol propionate, betamethasone dipropionate (BDP) compared to petrolatum in 16 healthy volunteers using ultrasound, optical coherence tomography, confocal laser scanning microscopy, multiphoton tomography (MPT) and resonance Raman spectroscopy over 4 weeks [70▪]. They could show a reduction in epidermal and dermal skin thickness in GC on the volar forearm. MPT analysis showed an increased epidermal cell density and reduced cell size and nucleusto-cytoplasm ratio. A restructuration and compression of collagen fibres corresponding to atrophic changes, was reported [70▪].

The same group studied with a similar design the effect of 4 weeks of topical clobetasol proprionate, BDP or vehicle on stratum corneum lipids (collected by tape stripping and analysed by a high sensitivity liquid chromatography–mass spectrometry) [71▪]. Clobetasol proprionate caused a reduction in 98 lipids whereas BDP treatment only resulted in a significant change of 29 lipids. Most pronounced changes occurred among long chain, ester-linked, ceramide classes whereas other ceramide classes were less affected [71▪]. This finding explains, partially, the side effects of topical glucocorticosteroids on barrier-associated parameters.

CONCLUSION

We reviewed the current knowledge on the noninvasive assessment of morphological, functional and biochemical composition of the skin in atopic dermatitis. Classical biophysical methods such as the TEWL, stratum corneum hydration and surface pH are established tools. They are amended by novel in-vivo techniques such as Raman spectroscopy for skin composition analysis as well as reflectance spectroscopy and optical coherence tomography for the assessment of morphological changes of the skin. The different noninvasive tools to assess different aspects of skin in atopic dermatitis are summarized in Table 1.

Acknowledgements

None.

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest, regarding the content of this article.

Table 1. Summary of the noninvasive tools used in the different aspects of morphology, function, biochemical and immunological pathways

Morphology	
Morphological patterns	Reflectance spectroscopy
Morphological patterns	Optical coherence tomography
Morphological patterns	High definition ultrasound
Tape stripping	Transmition electron microscopy
Stratum corneum thickness	Raman spectroscopy
Function	
Barrier function	TEWL
Stratum corneum hydration	Capacitance, conductance
Microcirculation/blood flow	Laser Doppler, laser speckle
Redness,	Mexameter, chromameter
Pigmentation	Mexameter, chromameter
Surface pH	pH-meter
Biochemistry	
Stratum corneum lipids	TS + Lipidomic
Sebum lipids	TS or extraction
Natural moisturizing factors	Raman spectroscopy
Water profile	Raman spectroscopy
Protein	TS
Immunology	
Cytokines	Tape stripping + ELISA
Chemokines	Tape stripping + ELISA

TEWL, transepidermal water loss; TS, tape stripping.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Schade H, Marchionini A. The acid mantle of the skin (after gas chain measurement). Klin Wschr 1928; 7:12–14.
2. Kim BE, Leung DYM. Significance of skin barrier dysfunction in atopic dermatitis. Allergy Asthma Immunol Res 2018; 10:207–215.
3. Margolis JS, Abuabara K, Bilker W, *et al.* Persistence of mild to moderate atopic dermatitis. JAMA Dermatol 2014; 150:593–600.
4. Zheng T, Yu J, Oh MH, Zhu Z. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. Allergy Asthma Immunol Res 2011; 3:67–73.
5. 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 2006; 38:441–446.
6. Elias PM, Schmuth M. Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. Curr Opin Allergy Clin Immunol 2009; 9:437–446.
7. Amat F, Saint-Pierre P, Bourrat E, *et al.* Early-onset atopic dermatitis in children: which are the phenotypes at risk of asthma? Results from the ORCA cohort. PLoS One 2015; 10:e0131369.
8. Biagini Myers JM, Wang N, LeMasters GK, *et al.* Genetic and environmental risk factors for childhood eczema development and allergic sensitization in the CCAAPS cohort. J Invest Dermatol 2010; 130:430–437.
9. Darlenski R, Kazandjieva J, Fluhr JW, *et al.* Lactic acid sting test does not differentiate between facial and generalized skin functional impairment in sensitive skin in atopic dermatitis and rosacea. J Dermatol Sci 2014; 76:151–153.
10. Tang TS, Bieber T, Williams HC. Are the concepts of induction of remission and treatment of subclinical inflammation in atopic dermatitis clinically useful? J Allergy Clin Immunol 2014; 133:1615–1625; e1.
11. Qassem M, Kyriacou PA. Effectiveness of the DreamSkin (R) garment on relieving symptoms of eczema/dermatitis using electrical and spectroscopic methods: a case study. Conf Proc IEEE Eng Med Biol Soc 2014; 2014:3723–3726.
12. Astner S, Gonzalez E, Cheung A, *et al.* Pilot study on the sensitivity and specificity of in vivo reflectance confocal microscopy in the diagnosis of allergic contact dermatitis. J Am Acad Dermatol 2005; 53:986–992.
13. Astner S, Gonzalez E, Cheung AC, *et al.* Noninvasive evaluation of the kinetics of allergic and irritant contact dermatitis. J Invest Dermatol 2005; 124: 351–359.
14. Samhaber KT, Buhl T, Brauns B, *et al.* Morphologic criteria of vesiculobullous skin disorders by in vivo reflectance confocal microscopy. J Dtsch Dermatol Ges 2016; 14:797–805.
15. Gambichler T, Moussa G, Sand M, *et al.* Correlation between clinical scoring of allergic patch test reactions and optical coherence tomography. J Biomed Opt 2005; 10:064030.
16. ■ Byers RA, Maiti R, Danby SG, *et al.* Sub-clinical assessment of atopic dermatitis severity using angiographic optical coherence tomography. Biomed Opt Express 2018; 9:2001–2017.

Pilot study with a novel technology to detect sub-clinical atopic dermatitis.

17. Aschoff R, Schmitt J, Knuschke P, *et al.* Evaluation of the atrophogenic potential of hydrocortisone 1% cream and pimecrolimus 1% cream in uninvolved forehead skin of patients with atopic dermatitis using optical coherence tomography. Exp Dermatol 2011; 20:832–836.
18. Boone M, Jemec GB, Del Marmol V. High-definition optical coherence tomography enables visualization of individual cells in healthy skin: comparison to reflectance confocal microscopy. Exp Dermatol 2012; 21:740–744.
19. Boone MA, Jemec GB, Del Marmol V. Differentiating allergic and irritant contact dermatitis by high-definition optical coherence tomography: a pilot study. Arch Dermatol Res 2015; 307:11–22.
20. Yatagai T, Shimauchi T, Yamaguchi H, *et al.* Sensitive skin is highly frequent in extrinsic atopic dermatitis and correlates with disease severity markers but not necessarily with skin barrier impairment. J Dermatol Sci 2018; 89:33–39.
21. ■ Simpson EL, Villarreal M, Jepson B, *et al.* Patients with atopic dermatitis colonized with staphylococcus aureus have a distinct phenotype and endotype. J Invest Dermatol 2018; doi: 10.1016/j.jid.2018.03.1517. [Epub ahead of print]

Endo- and phenotypisation sub-types of atopic dermatitis might lead to sub-type classification of this disease.

22. Hon KL, Kung J, Ng WG, *et al.* Are skin equipment for assessing childhood eczema any good? J Dermatolog Treat 2018; 1–15; doi: 10.1080/09546634.2018.1442551. [Epub ahead of print]
23. Horimukai K, Morita K, Narita M, *et al.* Transepidermal water loss measurement during infancy can predict the subsequent development of atopic dermatitis regardless of filaggrin mutations. Allergol Int 2016; 65:103–108.
24. Knor T, Meholjic-Fetahovic A, Mehmedagic A. Stratum corneum hydration and skin surface pH in patients with atopic dermatitis. Acta Dermatovenerol Croat 2011; 19:242–247.
25. Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol 2014; 134:792–799.
26. ■ Berardesca E, Mortillo S, Cameli N, *et al.* Efficacy of a shower cream and a lotion with skin-identical lipids in healthy subjects with atopic dry skin. J Cosmet Dermatol 2018; 17:477–483.

An important review on stratum corneum hydration.

27. Danby SG, Chittock J, Brown K, *et al.* The effect of tacrolimus compared with betamethasone valerate on the skin barrier in volunteers with quiescent atopic dermatitis. Br J Dermatol 2014; 170:914–921.
28. Berardesca E, Loden M, Serup J, *et al.* The revised EEMCO guidance for the in vivo measurement of water in the skin. Skin Res Technol 2018; 24: 351–358.
29. Schmid-Wendtner MH, Korting HC. The pH of the skin surface and its impact on the barrier function. Skin Pharmacol Physiol 2006; 19:296–302.
30. Fluhr JW, Elias PM. Stratum corneum pH: formation and function of the 'Acid Mantle'. Exogenous Dermatol 2002; 1:163–175.
31. Fluhr JW, Kao J, Jain M, *et al.* Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. J Invest Dermatol 2001; 117:44–51.
32. Krien PM, Kermici M. Evidence for the existence of a self-regulated enzymatic process within the human stratum corneum – an unexpected role for urocanic acid. J Invest Dermatol 2000; 115:414–420.
33. Behne MJ, Meyer JW, Hanson KM, *et al.* NHE1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. J Biol Chem 2002; 277: 47399–47406.
34. Mauro T, Holleran WM, Grayson S, *et al.* Barrier recovery is impeded at neutral pH, independent of ionic effects: implications for extracellular lipid processing. Arch Dermatol Res 1998; 290:215–222.
35. Hachem JP, Crumrine D, Fluhr J, *et al.* pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. J Invest Dermatol 2003; 121:345–353.
36. Hachem JP, Man MQ, Crumrine D, *et al.* Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. J Invest Dermatol 2005; 125:510–520.
37. Egelrud T, Hofer PA, Lundstrom A. Proteolytic degradation of desmosomes in plantar stratum corneum leads to cell dissociation in vitro. Acta Derm Venereol 1988; 68:93–97.
38. Komatsu N, Saijoh K, Sidiropoulos M, *et al.* Quantification of human tissue kallikreins in the stratum corneum: dependence on age and gender. J Invest Dermatol 2005; 125:1182–1189.
39. Proksch E. pH in nature, humans and skin. J Dermatol 2018; doi: 10.1111/1346-8138.14489. [Epub ahead of print]
40. Jang H, Matsuda A, Jung K, *et al.* Skin pH is the master switch of kallikrein 5-mediated skin barrier destruction in a murine atopic dermatitis model. J Invest Dermatol 2016; 136:127–135.
41. Vavrova K, Henkes D, Struver K, *et al.* Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways in a 3D skin construct. J Invest Dermatol 2014; 134:746–753.
42. ■■ Lee HJ, Lee NR, Kim BK, *et al.* Acidification of stratum corneum prevents the progression from atopic dermatitis to respiratory allergy. Exp Dermatol 2017; 26:66–72.

First time linking acidic skin pH and respiratory allergies.

43. Elias PM. Stratum corneum acidification: how and why? Exp Dermatol 2015; 24:179–180.
44. Lee NR, Lee HJ, Yoon NY, *et al.* Application of topical acids improves atopic dermatitis in murine model by enhancement of skin barrier functions regardless of the origin of acids. Ann Dermatol 2016; 28:690–696.
45. Sakai T, Hatano Y, Matsuda-Hirose H, *et al.* Combined benefits of a PAR2 inhibitor and stratum corneum acidification for murine atopic dermatitis. J Invest Dermatol 2016; 136:538–541.
46. Panther DJ, Jacob SE. The importance of acidification in atopic eczema: an underexplored avenue for treatment. J Clin Med 2015; 4:970–978.
47. Tkaczyk E. Innovations and developments in dermatologic noninvasive optical imaging and potential clinical applications. Acta Derm Venereol 2017; (Suppl 218):5–13.
48. Tsutsumi M, Fukuda M, Kumamoto J, *et al.* Abnormal morphology of blood vessels in erythematous skin from atopic dermatitis patients. Am J Dermatopathol 2016; 38:363–364.
49. Genovese A, Detoraki A, Granata F, *et al.* Angiogenesis, lymphangiogenesis and atopic dermatitis. Chem Immunol Allergy 2012; 96:50–60.
50. ■ Vaughn AR, Clark AK, Sivamani RK, Shi VY. Circadian rhythm in atopic dermatitis-Pathophysiology and implications for chronotherapy. Pediatr Dermatol 2018; 35:152–157.

Circadian rhythm in atopic dermatitis; importance for drug application.

51. Hazama Y, Maekawa T, Miki R, *et al.* Effect of physiological changes in the skin on systemic absorption of tacrolimus following topical application in rats. Biol Pharm Bull 2016; 39:343–352.
52. Firooz A, Gorouhi F, Davari P, *et al.* Comparison of hydration, sebum and pH values in clinically normal skin of patients with atopic dermatitis and healthy controls. Clin Exp Dermatol 2007; 32:321–322.

1528-4050 Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.

53. Furuichi M, Makino T, Matsunaga K, *et al.* The usefulness of sebum check film for measuring the secretion of sebum. Arch Dermatol Res 2010; 302:657–660.

54. Takigawa H, Nakagawa H, Kuzukawa M, *et al.* Deficient production of hexadecenoic acid in the skin is associated in part with the vulnerability of atopic dermatitis patients to colonization by staphylococcus aureus. Dermatology 2005; 211:240–248.

55. Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. Br J Dermatol 2008; 159:23–34.

56. Shimoda-Komatsu Y, Sato Y, Yamazaki Y, *et al.* A novel method to assess the potential role of sweating abnormalities in the pathogenesis of atopic dermatitis. Exp Dermatol 2018; 27:386–392.

57. Wruhs M, Gleiss A, Steiner A, Sator P. Quantity and quality of sweating in ■ atopic dermatitis. Arch Dermatol Res 2017; 309:787–793.
Quantification of sweat gland activity in atopic dermatitis.

58. Takahashi A, Murota H, Matsui S, *et al.* Decreased sudomotor function is involved in the formation of atopic eczema in the cubital fossa. Allergol Int 2013; 62:473–478.

59. Kohsaka T, Hiragun T, Ishii K, *et al.* Different hypersensitivities against ■ homologous proteins of MGL_1304 in patients with atopic dermatitis. Allergol Int 2018; 67:103–108.
Intimate mechanisms to sweat in atopic dermatitis.

60. Kaneko S, Murota H, Murata S, *et al.* Usefulness of sweat management for ■ patients with adult atopic dermatitis, regardless of sweat allergy: a pilot study. Biomed Res Int 2017; 2017:8746745.
Pilot study on auto-allergy to sweat in atopic dermatitis.

61. Simpson E, Bohling A, Bielfeldt S, *et al.* Improvement of skin barrier function in atopic dermatitis patients with a new moisturizer containing a ceramide precursor. J Dermatolog Treat 2013; 24:122–125.

62. Gonzalez FJ, Alda J, Moreno-Cruz B, *et al.* Use of Raman spectroscopy for the early detection of filaggrin-related atopic dermatitis. Skin Res Technol 2011; 17:45–50.

63. Gonzalez FJ, Valdes-Rodriguez R, Ramirez-Elias MG, *et al.* Noninvasive detection of filaggrin gene mutations using Raman spectroscopy. Biomed Opt Express 2011; 2:3363–3366.

64. Mischo M, von Kobyletzki LB, Brundermann E, *et al.* Similar appearance, different mechanisms: xerosis in HIV, atopic dermatitis and ageing. Exp Dermatol 2014; 23:446–448.

65. Mlitz V, Latreille J, Gardinier S, *et al.* Impact of filaggrin mutations on Raman spectra and biophysical properties of the stratum corneum in mild to moderate atopic dermatitis. J Eur Acad Dermatol Venereol 2012; 26:983–990.

66. Janssens M, van Smeden J, Puppels GJ, *et al.* Lipid to protein ratio plays an important role in the skin barrier function in patients with atopic eczema. Br J Dermatol 2014; 170:1248–1255.

67. O'Regan GM, Kemperman PM, Sandilands A, *et al.* Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. J Allergy Clin Immunol 2010; 126:574–580.

68. Kezic S, O'Regan GM, Lutter R, *et al.* Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. J Allergy Clin Immunol 2012; 129:1031–1039.

69. Sadowski T, Klose C, Gerl MJ, *et al.* Large-scale human skin lipidomics by ■ quantitative, high-throughput shotgun mass spectrometry. Sci Rep 2017; 7:43761.
Lipidomic depth profile of stratum corneum.

70. Jung S, Lademann J, Darvin ME, *et al.* In vivo characterization of structural ■ changes after topical application of glucocorticoids in healthy human skin. J Biomed Opt 2017; 22:76018.
Effect of topical glucocorticosteroids on skin physiology and collagen fiber.

71. Ropke MA, Alonso C, Jung S, *et al.* Effects of glucocorticoids on stratum ■ corneum lipids and function in human skin – a detailed lipidomic analysis. J Dermatol Sci 2017; 88:330–338.
Effect of topical glucocorticosteroids on lipidome.

Copyright © 2018 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.