Skin barrier in atopic dermatitis
Barreira cutânea na dermatite atópica

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Abstract: Research about the skin barrier and its properties has increased significantly since the 60s, with studies that indicated its resistance when isolated, as well as its particularities in relation to skin permeability. At the same time, description of Odland bodies helped to understand how stratum corneum stability is maintained. The “brick and mortar” model is the most accepted so far. In this analogy, the corneocytes are the bricks and the intercellular lipids are the mortar. Currently, there is concrete evidence that the stratum corneum is an active metabolic structure that holds adaptive functions, interacting dynamically with the underlying epidermal layers. The skin barrier also plays a role in the inflammatory response through melanocyte activation, angiogenesis, and fibroplasia. The intensity of this response will essentially depend on the severity of the injury. Skin barrier abnormalities in atopic dermatitis are clinically observed by the presence of dry skin, a common and significant symptom which constitutes a diagnostic and monitoring parameter. The stratum corneum hydration level and transepidermal water loss are associated with the level of damage to the barrier, representing biophysical parameters. These parameters help doctors monitor patients in a less invasive and more sensitive manner.

Keywords: Dermatitis, atopic; Insensible; Keratinocytes; Water loss

Resumo: O estudo da barreira cutânea e de suas propriedades ganhou impulso a partir da década de 60, com estudos que apontaram sua resistência de forma isolada e suas propriedades com relação à permeação cutânea. Paralelamente, a descrição dos corpos de Odland auxiliou a compreensão da manutenção da estabilidade da camada córnea. O modelo brick & mortar, em que os corneócitos são os tijolos e o cimento são os lípidos intercelulares, é o mais aceito, até o momento. Atualmente, há evidências consistentes de que o estrato córneo é uma estrutura metabolicamente ativa e exerce funções adaptativas. A barreira cutânea também tem um papel na resposta inflamatória, com ativação de melanócitos, angiogênese e fibroplasia, cuja intensidade depende, basicamente, da intensidade da agressão. As anormalidades da barreira cutânea da dermatite atópica são clinicamente observáveis pela presença de pele seca, achado muito frequente e significativo, que constitui parâmetro diagnóstico e de acompanhamento. O grau de hidratação da camada córnea, assim como a perda de água transepidérmica (transepidermal water loss - TEWL), estão relacionados com o grau de dano à barreira, constituindo parâmetros biofísicos que permitem acompanhar os pacientes de maneira não invasiva e com maior grau de sensibilidade.

Palavras-chave: Dermatite atópica; Queratinócitos; Perda insensível de água

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INTRODUCTION

Atopic dermatitis (AD) is a chronic disease with evolution in outbreaks that predominates in infancy. Its main symptom is pruritus of variable intensity and its main signs are skin xerosis and eczematous lesions. The stimulus for an abnormal response is often external, due to alteration of the skin barrier: there is the development of xerosis, with abnormalities in the stratum corneum, and increase of transepidermic water loss, which also cause an abnormal IL-4 metabolism.

Epidemiologically, AD is one of the most frequent inflammatory dermatoses in infancy, normally with onset in the first years of life. Of the children who develop AD, 50% manifest the disease within the first year of life and 30%, from the first to the fifth year.

Prevalence of AD has increased over the last years, and environmental factors seem to play an important role in this growth.

In AD, skin xerosis, a terminology used to describe dry skin, is a very frequent and significant symptom: because it is the clinical expression of the skin barrier abnormality shown by these patients, it is a diagnostic and monitoring parameter.

In the physiopathology of AD, impairment of the skin barrier is associated with a reduction in the levels of ceramide and in the production of profilaggrin, with greater transepidermic water loss (TEWL) and higher predisposition to aggression, which are the trigger for inflammation.

Instrumental evaluations of morphological parameters have been investigated to obtain objective reproducible measurements of the skin barrier conditions. Non-invasive techniques have been developed and validated for a few specific parameters.

Two methods that employ instrumental analysis may be used for skin barrier evaluation in AD:

Measurement of the hydric content of the corneal layer (corneometry)
Measurement of transepidermic water loss (TEWL)

These measures identify subtle changes, non-perceptible clinically, with reproducibility in standardized conditions.

3.1. Skin barrier

The idea that the skin barrier would be a simple “mantle” that separated the internal medium from the environment underwent radical changes over the last 50 years. Until the 1960s, it was thought that the skin barrier was in fact in the upper portion of the granulous layer and was not formed by the stratum corneum. The first works to modify this belief were those conducted by Cristopher and Kligman, who analyzed the stratum corneum in isolation and showed its resistance. At the same time, studies by Blank and Scheuplein and Blank showed the particularities of the stratum corneum (SC) permeability, whose infiltration is determined by the chemical characteristics of the molecule and thickness of the SC, in addition to its humidity level, according to what was detailed by Sato et al. (2002).

Simultaneously, Odland described the organelles that are named after him, also called lamellar bodies, whose structure contains a mixture of ceramides, cholesterol, and free fatty acids. They play a role in the formation of the lipid component of the skin barrier (through exocytosis) and in the maintenance of the stratum corneum stability.

In 1975, Michaels et al. suggested a schematic model to explain the permeability of the stratum corneum, called “brick & mortar”, where the corneocytes are the bricks and the lips are the mortar. This model was reviewed and validated by Jonhson et al. in 1997. Today this is viewed as the most appropriate model for the understanding of cellular arrangement and the winding ways of skin permeability (Figure 1).

The stratum corneum is a metabolically active structure and it also has adaptive functions, with great interaction with the subjacent epidermal layers.

Physiologically, the stratum corneum is formed by a sequence of events:

1. The keratinocyte cellular membrane of the granulous layer becomes more permeable to ions, especially calcium, which activate peptidases and convert pro-filaggrin into filaggrin: filaggrin is an intermediate filament-associated protein that exists in the granules of kerato-hialine and activates the enzymes trigliceridadase and aggregates keratin filaments with macrofibrils; next, this protein is degraded to free aminoacids that will later be used in the constitution of corneocytes.

![Figure 1: "Brick and mortar" pattern of the stratum corneum (skin barrier)](source: Michaels AS, et al)
of the natural moisturizing factor or converted into urocanic acid or pyrrolidone carboxylic acid (PCA). 18

Filaggrin is responsible for aggregating keratin and other proteins in the superficial layers of the epidermis to form the stratum corneum; 19 the process of conversion of profilaggrin into filaggrin maintains the integrity of the epidermis (Figure 2).

2. With the degeneration of the cellular nucleus, cells become flat and keratin molecules align in parallel, creating a cornified envelope, connected to extracellular lipids. 20 The cohesion power of this layer depends upon the formation of covalent connections of lysine glutamine, where precursor proteins are incorporated into keratin: involucrin, small proline-rich peptides (SPRP), cornifin, loricrin, keratoline, and desmosomal proteins such as envoplakin and perilplakin. 21

3. Lamellar bodies, originated in the granulous layer, also contribute to the formation of the lipid matrix in which corneocytes are located (Figure 3). 22,23

Studies by Elias et al. 17,24,25,26 showed that any disturbance of the skin barrier unchains a repair response that may last for days or hours, based on the intensity of the stimulus. Initially, secretion of a pool of pre-formed lamellar bodies occurs, followed by an increase in the synthesis of cholesterol and free fatty acids and ceramides; at the same time, there is an increase in enzymes and levels of RNA relative to these enzymes, with primary activation by phosphorylation of HMG-CoA reductase and of sterol regulatory element binding proteins (SREBPs) as regulators of the synthesis of cholesterol and epidermal fatty acids. In addition, an increase in the synthesis of epidermal DNA is observed.

Regarding the immunological aspect, there is release of IL1 alpha pools and increase in the synthesis of proteins constituent of cytokines, with the generation of intracellular adhesion molecules (ICAM), and increase of TNF concentration and granulocytemacrophage colony stimulating factor (GM-CSF), with consequent activation of Langerhans cells. These phenomena unchain an inflammatory response and activate melanocytes, angiogenesis, and fibroplasia, 27 whose intensity will depend essentially on the intensity of the aggression (Figure 4).

3.2. Skin barrier and atopic dermatitis

Atopic dermatitis is a chronic eczematous dermatitis, characterized by Th2 and Th1 reaction patterns against environmental allergens. In AD, skin
Xerosis is a very common and significant finding; because it is the clinical expression of the skin barrier abnormality presented by these patients, it is a diagnostic and monitoring parameter. Studies that correlate skin barrier alterations and AD are summarized in Chart 1.

A study by Bohme et al. 28, conducted with 221 atopic children, clearly shows the importance of this small criterion, observed in their sample in 100% of the patients.

Xerosis is associated with a deficiency in the function of the epidermal barrier. 29,30 Alterations of the barrier function of the stratum corneum are present not only in the affected skin, but also in the apparently normal skin, during the activity of dermatitis.

Di Nardo et al. 31 showed a significant reduction in the levels of ceramides 1 and 3 in the stratum corneum; complementarily, a study by Pilgram et al. 32 demonstrated a structural imbalance of the extracellular lipid matrix; Murata et al. 33 reported an increase in glucosylceramide/esfingomielina deacylase levels as a mechanism of ceramide reduction, occurring in the atopic affected or normal skin. Later, Fartash and Diepgen 34 and Fartash 35 described an alteration in the extrusion of lamellar bodies in the dry skin of atopic patients.

Nonetheless, the organization and maturation of corneocytes are normal in the dry skin of the atopic patient. 36 Pastore et al. postulated that keratinocytes are capable of regulating the immune response in AD, activating, through an increase in the expression of granulocyte-macrophage colony stimulating factor (GM-CSF), epidermal and dermal dendritic cells. 37

Metabolic alterations of esfingomielina, which lead to a reduction in the levels of ceramides (in particular ceramide 1), also have an important and negative influence on the barrier function, as shown in a study by Hara et al. in 2000. 38

A review by Simpson and Hanifin 39 and studies by Choi and Maibach 40 emphasized the growing interest in the impact of the skin barrier on the evolution of AD, with a strong evidence of its abnormalities.

A recent study by Farwanah et al., 2005, 41 comparing psoriasis and AD patients with normal individuals, did not find a significant deficiency in the amount of ceramides in the two groups. Therefore, these authors believe that the parameter ceramide cannot be considered a diagnostic criterion.

Results from another study conducted in the same year, by Lebwohl and Hermann 42, dispute these findings as even skin without an apparent lesion, but with xerosis, shows alterations related to a reduction in ceramide levels.

The primary barrier damage of atopic dermatitis facilitates the action of irritants and reduces the pruritus threshold, helping the trauma caused by itching and consequent barrier injury. Any trauma to the barrier activates a cascade of cytokines secreted by keratinocytes, aggravating and perpetuating the inflammatory process. 43,44

Many mediating agents are involved in the genesis of the inflamed skin pruritus; histamine is essential, but not the only one: cytokines, prostaglandines, tachykinins, P substance, and others also play a role in the development of the symptom. 45

Another factor is the modulation of sensory nerves in the presentation of antigens and skin inflammation. There is evidence that pruritus is a complex sensation, influenced not only by the intensity of the stimulus or severity of the atopic disease, but also by central neurologic stimuli, according to findings by Heyer et al. 46 Studies by Seidenari and Giusti attempted to correlate the degree of pruritus with instrumental measurements of the barrier function; these authors observed a progressive reduction of skin hydration values (corneometry) based on pruritus

**Figure 4:** Aggression to the barrier and development of the inflammatory response

- Damage to the barrier
- Epidermal injury
- Presentation of pre-formed
  - Generation and presentation of IL1a, TNFa, GM-CSG, IL6
  - Chemotactic and activating cytokines (e.g.:IL 8)
  - Migration and activation of inflammatory cells
  - Macrophage-derived cytokines

Adapted source: Elias PM, et al. 20
Conclusion

Significant reduction of ceramides 1 and 3 in the stratum corneum
Alterations of the intercellular lipid organization
Reduction of the levels of ceramides in the atopic affected or normal skin through an increase in the expression of sphingomyelin acylase
Alteration of lamellar bodies extrusion in the atopic skin with xerosis
Regulation by the immune response keratinocytes in AD, with greater activity of dermal and epidermal dendritic cells
Higher expression of sphingomyelin deacylase is responsible for ceramide deficiency in the skin barrier of the atopic patient
Increased activity of ceramidase, sphingomyelin deacylase, and glucosylceramide deacylase in AD patients

Methodology

Thin layer chromatography of corneal biopsies with cyanocrylate glue
Skin biopsy samples examined using electron microscopy
Stratum corneum from biopsy samples of AD patients, evaluated by liquid chromatography
Comparative study of biopsies of atopic, psoriatic patients, with lamellar ichthyosis, by ruthenium tetroxide fixation
Keratinocyte culture under analysis of a reporter gene (chloramphenicol acetyl transferase)
Enzymatic measurements of the stratum corneum of AD patients compared with contact dermatitis subjects
Enzymatic measurements of the stratum corneum of atopic patients

Biophysical parameters for the evaluation of skin barrier in AD:

Clinical examination is an essential tool to the dermatologist for the identification of AD. However, the subjective component may interfere with evaluations for research purposes.

There are still subclinical skin alterations, subtle to inspection, in which there is injury or damage to the barrier that is undetectable in a routine clinical exam. In the 1960s, laboratory studies about skin physiology, in particular by a German group, analyzed mechanical properties of the structure of the skin. A study by Ridge and Wright, of 1996, was one of the first publications.

The practical applicability of these technologies to measure skin parameters, such as water amount, microcirculation, pigmentation, and elasticity, came with the creation of standardized equipment, which made the establishment of methods and reproducibility of measurements possible.

These methodologies, generically named skin bioengineering techniques, proved to be useful in the evaluation of skin diseases, in addition to providing elements for the consideration of treatments to these disorders.
In the 1980s, a study of biophysical parameters by the groups of Berardesca (Europe) and Maibach (USA) helped the development of bioengineering equipment to measure a few skin parameters.

This equipment allowed the development of methods with the following characteristics:

- They are not invasive;
- They offer objective measurements with phenomena quantification;
- Standardization of places, techniques and environmental conditions;
- Evaluations without interfering with treatments or with the spontaneous course of the patient's condition.

The standardization of environmental conditions for the collection of measurements is essential so that they can be reproduced.

The correlation between the intensity of AD and the biophysical measurements of lesioned skin has been shown in a study with children and adolescents, conducted by Kim et al.

A significant correlation between these measurements in normal skin was confirmed by another study conducted that same year, by Holm et al. This study attempted to validate hydration parameters, TEWL, as well as ultrasonography and Doppler in the evaluation of AD in 101 atopic patients and 30 healthy control individuals. Although the authors do not consider these measurements the gold standard for the evaluation of the prognosis of AD, their results showed significant differences between the atopic lesioned skin, normal skin and control group, suggesting a correlation with the level of activity of the disease. This work also shows that even measurements of apparently normal skin are useful for the evaluation of the intensity of the condition.

In 2008, two groups of researchers, Hon et al. and Gupta et al., showed a correlation between TEWL and SCORAD in children, proving that there is an association between injury to the skin barrier measured by TEWL and the clinical intensity of the disease.

3.3.2 Functional evaluation of the skin barrier: transepidermal water loss

Transepidermal water loss (TEWL) expresses measurements of water diffusion through the skin and it is an important parameter of the skin barrier integrity.

The method described by Nilsson in 1997 uses an open chamber with two sensors in different levels and obtains the degree of evaporation based on the gradient of these measurements. With environmental (humidity and temperature) and patient standardization (resting for acclimation 15 minutes on average before measurement, to reduce the effects of perspiration and vasodilation caused by physical activity), measures can be obtained with reproducibility.

Equipment items were developed based on this principle to obtain these measurements.

In 1995, Barel and Clarys studied equipment for the calculation of transepidermal water loss (Evaporimeter® and Tewameter®). The measurement technique was the evaporation gradient; they collected measurements in normal skin and skin with irritation induced by stripping, in standard laboratory conditions. They achieved a good correlation and reproducibility of the results with this validation.

Transepidermal water loss (TEWL) shows normal levels according to the area of the body. In the trunk, for instance, there is spontaneous water loss through the corneal layer in the amount of 3-6 g/h/m²; in the face, values range from 1 to 15 g/h/m². These variations are due to the thickness of the stratum corneum and to the dermal microvasculature.

After the stratum corneum has been injured, the loss may reach 70 g/h/m². This is a convenient way to measure the extent of the barrier disfunction and constitutes an important instructive element for its evaluation.

The Tewameter® used is the TM 300 model, by Courage & Khazaka, whose measurements are considered accurate and reproducible if performed in a proper environment by trained personnel. According to Miteva et al., who evaluated the available methods of measurement and calibration in standard conditions, concluded that this equipment can achieve reliable measurements that can be reproduced in standard experimental conditions.

Even in the recovery phase, the atopic patient shows dryness or roughness of the skin with increased TEWL. This increase occurs both in the affected and normal skin of atopic patients, as shown by Seidenari and Giusti in a study with 66 atopic and 21 healthy children (without atopy or xerosis).

Measurements obtained of each group were significantly higher for the atopic patients when compared to the control group.

Another study by the same authors with children shows that even without active lesions, the atopic child has a significant increase of TEWL (Chart 2). A total of 200 atopic and 45 non-atopic children (control group) participated in the study.

The averages obtained for each group were significantly higher for the atopic patients, when compared with the control group (p<0.05).

With these findings, the authors concluded that the measurement of TEWL is a functional marker of AD, and that it can vary based on the presence of lesions, even in normal skin.

TEWL tends to normalize in the normal skin of atopic individuals in remission of AD. Measurements
of water loss and hydration (capacitance) tend to vary based on the course of the disease, suggesting recovery of the skin barrier or that these alterations are reversible. A study with normal individuals without clinical signs of AD shows normal levels of TEWL in the forearm, according to Loffler and Effendy. Patients without lesions for over two years showed similar values of TEWL in the forearm to those of non-atopic individuals, according to a study by Agner. Simultaneously, Conti et al. studied a probable correlation between the biophysical parameters and the presence of other atopies, but they did not find any change in the measurements associated with respiratory alterations.

3.3.3 Evaluation of water content in the corneal layer: capacitance

The amount of water in the corneal layer may influence the skin barrier function: greater hydration increases percutaneous absorption.

The amount of water retained in the stratum corneum depends on the capacity of water retention, which maintains the skin soft and flexible, even in dry environmental conditions; this water also helps enzymatic reactions in the maturation and scaling of corneocytes.

Water reduction leads to fissures in the stratum corneum, which allow greater penetration of heavier molecular substances, including allergens and microorganisms.

Hydration of the skin surface may be measured by electrical methods. Conductance measurements, described by Watanabe, Tagami et al. assess only the surface, while other capacitance measurements go deeper. Similar results of comparative studies of methodologies were also found by Agner and Serup, in 1998, and by Fluhr et al. in 1999.

Capacitance values measured by the Corneometer were correlated with degrees of dry skin clinically observed in a multicentric study conducted in Germany with 349 individuals in 6 places (Chart 3). Loden et al. found significantly lower capacitance values in patients with AD, especially those with dry skin observed clinically and with higher values of TEWL.

The same author established a correlation between clinical findings about the skin of patients with AD and the intensity of dryness, measurable through capacitance.

As TEWL, capacitance also changes according to the activity of the disease. To show this, Werner evaluated 40 atopic patients: 20 of them with clinically dry skin and 20 with clinically normal skin. The author found a higher average of measurements for the normal skin, with a significant value difference (p<0.01).

Biophysical methods have had various applications in AD: they provide elements to measure the improvement of the barrier function and water content in the corneum stratum after treatment with drugs and moisturizers.

The correlation between non-invasive biophysical parameters of the skin barrier and signs and symptoms of AD has been investigated. Sugarman et al. developed a scale based on measurements of corneometry and TEWL, added to the clinical notes of the SCORAD scale, to determine the severity of the condition. However, studies that investigate the correlation between measurements of water content in the corneal layer and TEWL and clinical, epidemiological, and biochemical parameters are still limited in the literature.

Determination of the hydric content of the corneal layer can be accomplished through electrical measurements of the skin surface. The mechanism is the following: water has a higher dielectric constant than the skin. An increase in the hydric content will increase capacitance values, that is, the capacity to maintain a gradient of electrical charge. The equipment that allows this measurement is based on dielectric constant changes, which in turn alter the capacitance.

The measurement of TEWL is based on the passive diffusion of water through the stratum corneum, whose gradient is measured by the open probe of the equipment. In both situations, the accuracy of the measurements and their reproducibility will essentially depend on a standardized environment in relation to temperature and humidity, excluding variables such as perspiration and products applied to the skin.

There is a reduction in the capacitance values of the atopic skin compared to the non-atopic, as well as a significantly greater TEWL in the skin of these atopic children, non-active, and non-atopic

<table>
<thead>
<tr>
<th></th>
<th>Non-eczematous skin of atopic individuals with activity</th>
<th>Non-eczematous skin of atopic individuals without activity</th>
<th>Non-atopic individuals (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9,02 ± 5,32</td>
<td>7,56 ± 4,54</td>
<td>5,38 ± 2,96</td>
</tr>
</tbody>
</table>

Adapted source: Giusti G, et al. 65
patients in comparison with normal skin. The authors also noticed that the presence of respiratory atopy did not lead to significant alterations of these measurements. \cite{85,86}

**CONCLUSION**

Atopic dermatitis is a chronic disease in which abnormalities in the immune response and constitution of the skin barrier play a fundamental role in the outbreaks of the disorder. The skin barrier may show genetic alterations, but it may also suffer influence of neurologic, immunological, and environmental order.

Changes in the skin barrier affect its function; when its barrier function is damaged, the condition tends to worsen, as the pruritus threshold falls, leading to a concomitant aggravation of the inflammatory process.

This inflammatory condition appears to be present in the atopic patient even in areas of clinically normal skin, since the biophysical measurements of TEWL and corneometry are altered in these areas. Some studies have shown a significantly increased TEWL in the non-lesioned skin of the atopic patient, attributed to a disturbance in the maturation of lamellar bodies.

Biophysical measurements of the non-lesioned skin of AD patients appear to have a correlation with the clinical severity of the disease. With remission of the skin condition, the tendency is the normalization of measurements, according to the literature.

This finding may show that the biophysical parameters of evaluation of the skin barrier may be a reliable indicator of AD activity, even if, at the time of the clinical examination, there is absence of detectable lesions. 

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**CHART 3: Determination of the degree of xerosis in the skin surface using the corneometer**

<table>
<thead>
<tr>
<th>Clinical degree of xerosis</th>
<th>Measurement (corneometer units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (without clinical xerosis)</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Dry</td>
<td>40-30</td>
</tr>
<tr>
<td>Very dry</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

Adapted source: Werner Y \cite{75}

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REFERENCES

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