SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Examination of the values of diagnostic tests in immunological disorders

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The Examination takes place at the Libraryof Department of Dermatology, Faculty of Medicine, University of Debrecen, at 11:00, on 22th April, 2026.

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, at 13:00, on 22th of April, 2026.

1. INTRODUCTION

1.1 Urticaria

Urticaria is an itchy skin condition characterized by the appearance of hives, affecting up to 10% of the population. It can present with hives (wheals), angioedema, or a combination of both. Other conditions may also be associated with urticaria and/or angioedema, such as anaphylaxis, autoinflammatory syndromes, urticarial vasculitis, or hereditary angioedemas, which should be differentiated from urticarias based on their etiology. An urtica (wheal) is a well-defined, superficial intercellular oedema in the papillary dermis that typically disappear from the skin surface without a trace within 30 minutes to 24 hours. Angioedema is a deeper swelling caused by fluid accumulation in the deeper layers of the dermis and subcutis. It presents as a sudden swelling that may be erythematous or skin-coloured. The oedema is typically stinging, burning, sometimes painful, and less itchy than hives. Its resolution takes longer than urticaria, sometimes up to 72 hours. Urticaria can be classified into two main groups. Based on its duration, acute urticaria, which typically resolves within a few days but definitely within 6 weeks, and chronic urticaria, which persists for more than 6 weeks, can be distinguished. Chronic urticaria can be further divided into chronic spontaneous urticaria (CSU) and chronic inducible urticaria (CIndU).

1.2 Chronic spontaneous urticaria (CSU)

CSU, formerly known as chronic idiopathic urticaria, refers to hives that have persisted for more than 6 weeks and are triggered by unknown causes. Approximately 1% of the population suffers from CSU, though some literature suggests its prevalence is increasing. CSU can affect both children and adults; in adults, it occurs more frequently in women. The average duration of the disease is about 3-5 years. However, in severe cases — especially when the patient experiences recurrent angioedema, has coexisting CIndU, or has a positive autologous serum skin test (ASST) — the condition may last even longer. CSU significantly impairs patients' quality of life through the appearance of widespread, itchy or burning wheals and episodes of angioedema. It is often associated with other autoimmune diseases, most commonly autoimmune thyroiditis or vitiligo.

Like other forms of urticaria, CSU is triggered by the activation and degranulation of mast cells and basophil granulocytes. Additionally, eosinophil granulocytes, T and B lymphocytes, and epithelial and endothelial cells are also involved in the process. However, the exact

pathomechanism of the disease remains unknown. So far, signaling defects and an autoimmune background have been identified as contributing factors in this disease group.

1.2.1 Pathomechanism and clinical features of chronic autoimmune urticaria (AIU)

According to the classical Type IIb hypersensitivity reaction, in AIU, autoantibodies of the IgG, IgA, and/or IgM type are produced against IgE or the FcεRI. Circulating autoantibodies can be detected in approximately 40% of CSU patients. Autoantibodies targeting FcεRI are more common than those directed against IgE. The binding of anti-FcεRI autoantibodies to their receptor leads to abnormal, continuous stimulation and subsequent degranulation of mast cells and basophils. On the other hand, IgG autoantibodies against IgE can bind to the receptor and cross-link with IgE on the surface of mast cells and basophils, also resulting in their activation and degranulation.

More recently, it has also been demonstrated that IgE-type autoantibodies can bind to FcεRI and induce pathological activation. This form of CSU is referred to in the literature as autoallergic CSU. Among CSU patients, IgE autoantibodies most commonly target thyroid peroxidase (TPO), but antibodies against eosinophil peroxidase are also frequently observed. Additionally, IgE autoantibodies have been identified against double-stranded deoxyribonucleic acid, IL-24, tissue factor, eosinophil cationic protein, FcεRI, and thyroglobulin.

An increased prevalence of the human leucocyte antigen (HLA) – DR4 allele has been reported among CSU patients. This allele is associated with several autoimmune diseases, such as rheumatoid arthritis, type 1 diabetes mellitus, and multiple sclerosis. However, there is no clinical evidence that CSU itself is associated with these specific diseases. Later, the association with HLA-DR4 could not be confirmed in larger populations, although other HLA associations (such as HLA-DR9, HLA-DR12, and HLA-DRB1) have been identified by several research groups.

1.2.2 Diagnosis of AIU and its challenges

The diagnosis of AIU is challenging. According to current diagnostic and therapeutic guidelines, it is not necessary to establish the diagnosis of AIU specifically; rather, it is sufficient to classify the patient with chronic urticaria (CU) as having either inducible or spontaneous urticaria. Once chronic inducible urticaria has been excluded, routine testing typically includes only inflammatory markers — C-reactive protein (CRP) and/or erythrocyte sedimentation rate — as well as anti-TPO antibodies and total IgE levels. If a drug-induced

origin is suspected, discontinuation of the medication is recommended. Further diagnostic steps may include ruling out infections, detection of functional autoantibodies, thyroid function testing, allergy testing (e.g., skin prick tests, allergen avoidance), measurement of serum tryptase in the case of severe systemic reactions, or skin biopsy in case of diagnostic uncertainty.

One method previously used in the diagnosis of AIU, although no longer included in current guidelines, is the ASST. The test was first used by Grattan et al., and later standardized by Sabroe and colleagues The sensitivity of the test is approximately 70%, and its specificity is around 80%. A positive result may indicate the presence of functional autoantibodies against FcεRIα, anti-IgE autoantibodies, or other, yet unidentified serum factors. The advantages of this method are its speed, simplicity, and low cost; however, it is not sufficient on its own to confirm the diagnosis of AIU and must be supplemented with confirmatory testing. Some authors have proposed immunoassays [e.g., immunoblot and enzyme-linked immunosorbent assay (ELISA)] for detecting anti-FcεRIα autoantibodies. However, these binding assays are unable to distinguish between functional and non-functional autoantibodies, they are time-consuming, and they carry a risk of false-positive and false-negative results, limiting their utility as confirmatory tests. The latest European guideline recommends detecting functional autoantibodies, with the basophil histamine release assay (BHRA) considered the gold standard. BHRA utilizes the patient's whole serum, which means that cytokines and complement factors present in the serum may influence histamine release from basophils. It is also known that basophils from different donors can produce variable results. Furthermore, basophils differ in phenotype and behavior from mast cells, which are the primary effector cells in urticaria. In addition to BHRA, the literature also supports the use of basophil CD63 expression assays as confirmatory functional tests in the diagnostics of AIU. This method can serve as an alternative to histamine release assays in laboratories equipped with flow cytometry. However, all confirmatory tests mentioned are technically demanding, time-consuming, and costly, and many laboratories lack the infrastructure for their routine implementation. Therefore, they do not facilitate everyday clinical practice.

1.2.3 Treatment of AIU

The treatment of AIU is identical to that of CSU; however, AIU tends to respond less effectively to standard therapies.

The first-line treatment consists of second-generation H1 antihistamines (AH). If adequate symptom control is not achieved within 2–4 weeks at the standard dose, the dosage may be

increased up to four times the standard amount. If high-dose AHs alone are insufficient, initiation of omalizumab therapy should be considered. Omalizumab is a humanized monoclonal antibody targeting IgE. Its mechanism of action in CSU is not yet fully elucidated; it is assumed that, in addition to binding circulating IgE, it also downregulates FcεRI receptors on the surface of basophil cells, thereby reducing their activation and histamine release. If the standard omalizumab dose (300 mg every 4 weeks) proves ineffective, the dose may be increased and/or the administration interval shortened—up to 600 mg every 2 weeks—after obtaining special regulatory approval in Hungary. If no improvement is observed within 6 months, treatment should be complemented with cyclosporine in combination with second-generation AHs, at a maximum dose of 5 mg/kg body weight.

In rare, therapy-resistant cases, short-term corticosteroid treatment may be administered. In selected individual cases, sulfasalazine, methotrexate, interferon, plasmapheresis, phototherapy, or intravenous immunoglobulin may also be attempted; however, their use is supported only by case reports in the literature.

1.3 Atopic Dermatitis (AD)

1.3.1 Epidemiology and pathogenesis of AD

AD is a common, chronic inflammatory skin disease with a high prevalence—affecting 15–20% of children, and up to 10% of adults. While AD can develop at any age, it typically presents between 3 and 6 months of age. The condition often resolves by 4–5 years of age, but in more severe cases, it may persist into adulthood. In addition to early-onset disease, adult- or even AD in the elderly is also recognized. It is well known that AD may be associated with other atopic diseases such as food allergies, allergic rhinitis (AR), and asthma, which may appear sequentially or overlap within the same individual — a phenomenon referred to as the atopic march. This association is especially prevalent in early-onset AD and is less characteristic of adult-onset disease. AD is not only associated with atopic diseases but also with psychiatric conditions (depression, anxiety, and even suicidal ideation), autoimmune and other immunological disorders (vitiligo, chronic urticaria, celiac disease, inflammatory bowel diseases, rheumatoid arthritis, systemic lupus erythematosus), infectious diseases (herpes, chickenpox, common warts, plantar warts), contact dermatitis, and, according to some studies, with cardiovascular diseases, malignancies (such as squamous cell carcinoma), and osteoporosis.

AD is a multifactorial disease, with genetic, environmental, and immunological factors contributing to its pathogenesis. A central role is played by epidermal barrier dysfunction in

combination with predominantly Th2- and Th22-skewed inflammation. Barrier dysfunction may result from genetic mutations, environmental insults, or the inflammatory process itself. The most important structural protein of the skin barrier is filaggrin (FLG). Loss-of-function mutations in the *FLG* gene (e.g., R501X, 2282del4) and acquired FLG downregulation due to Th2 cytokines are both associated with significant impairment of the barrier. These *FLG* mutations are found in 14–56% of patients with AD. Other structural components of the barrier, such as loricrin and involucrin, may also be genetically or environmentally altered in AD. Disruption of the lipid matrix can occur through environmental exposure (e.g., detergents, soaps), genetic mutations (e.g., *SPINK5*, encoding serine protease inhibitor Kazal-type 5), and tight junction impairment, either via inflammation (Th17-mediated) or mutations in claudin-1 or claudin-2. These alterations collectively reduce barrier integrity and function.

Both innate and adaptive immune responses are dysregulated in AD. Within the innate immune system, keratinocyte-derived cytokines, notably thymic stromal lymphopoietin (TSLP), are pivotal in initiating Th2-type inflammation. TSLP is an IL-7-like cytokine produced by barrier-forming epithelial cells (skin, lung, gut). Environmental stimuli activate pattern recognition receptors, especially toll-like receptor 2 (TLR2), on keratinocytes, leading to increased TSLP production. TSLP activates dendritic cells (DCs), natural killer (NK) cells, and mast cells. DCs promote the differentiation of naive T cells into Th2 cells, which produce IL-4, IL-5, and IL-13, further enhancing TSLP production in a feed-forward loop. Keratinocyte-derived IL-25 and IL-33 activate type 2 innate lymphoid cells, further amplifying Th2 inflammation. In response to danger signals, keratinocytes also produce antimicrobial peptides (AMPs); however, their expression is downregulated by Th2 cytokines, compromising antimicrobial defense. As a result, the skin becomes susceptible to colonization by pathogens such as *Staphylococcus aureus*, further damaging the barrier. DC subsets play distinct roles in AD: inflammatory epidermal DCs promote Th2 differentiation, while Langerhans cells (myeloid epidermal DCs) contribute to allergic sensitization. In the Th2 milieu, Langerhans cells fail to produce IL-10, a key anti-inflammatory cytokine, which facilitates chronic inflammation. Not all DC subsets are activated in AD—plasmacytoid DCs undergo apoptosis in Th2 conditions, reducing their numbers and increasing susceptibility to viral infections and eczema herpeticum.

Among innate cells, NK cells are reduced in number in AD, yet when activated by TSLP, they contribute to the Th2 immune response, in collaboration with myeloid DCs.

The adaptive immune response in AD is skewed toward Th2 and Th22, with lesser contributions from Th1 and Th17 cells, and this profile is present in both acute and chronic lesions. Th2 (IL-4, IL-5, IL-13, IL-31) and Th22 (IL-22) cytokines contribute to barrier damage by reducing

FLG and claudin expression, inhibiting keratinocyte differentiation, and enhancing S. aureus colonization through AMP suppression. IL-31 plays a key role in itch perception in AD. Th17 cells are also present in AD lesions, albeit to a lesser extent. IL-22 expression is more prominent than IL-17, and Th17 cells are thought to promote epidermal hyperplasia and impair AMP production, increasing infection risk. Regulatory T cells (Tregs), specifically the CD4+CD25^brightFOXP3+ subset, are increased in AD and regulate allergen-specific immune responses. However, their immunomodulatory function is impaired in the presence of Staphylococcal enterotoxin B, contributing to persistent inflammation.

The skin microbiome is also altered in AD. Its composition can be affected by *FLG* mutations, environmental exposures (e.g., detergents, soaps), and topical treatments (corticosteroids, antibiotics). These factors reduce microbial diversity, allowing pathogenic species such as *S. aureus* to dominate. In AD, the compromised skin barrier and altered immune responses facilitate the overgrowth of such pathogens.

1.3.2 Diagnosis and clinical presentation of AD

Although several diagnostic criteria have been developed over the years for AD, the classic Hanifin and Rajka criteria in modified form remain the most widely used in clinical practice. According to these criteria, the diagnosis of AD requires the presence of pruritus along with eczema-like skin lesions characteristic of AD. Important criteria include: early onset, presence of atopy, xerosis. Associated clinical signs include: facial pallor, white dermographism, keratosis pilaris, palmar hyperlinearity, ichthyosis, ocular and periorbital changes, lichenification, excoriations, and perifollicular accentuation. There is no specific laboratory or *in vivo* test required to establish a diagnosis of AD. The diagnosis of AD can be established based on the clinical presentation. However, in cases where concomitant allergies are suspected, confirmatory tests such as skin prick testing (SPT), atopy patch testing (APT), standard patch testing, as well as total and specific IgE measurements may be considered. Additionally, patients with AD may present with elevated serum lactate dehydrogenase (LDH) levels and peripheral eosinophilia, though these findings are nonspecific and not diagnostic.

To assess disease severity, several scoring systems are available, the most widely used being: SCORing Atopic Dermatitis (SCORAD), Eczema Area and Severity Index (EASI), and the Investigator Global Assessment (IGA). These tools evaluate both the extent of the disease and the severity of hallmark eczematous features, allowing for standardized monitoring and treatment evaluation. The SCORAD assesses the extent of symptoms across all body regions and then evaluates the severity of erythema, infiltration, oozing/crusting, excoriation,

lichenification, and skin dryness. The latter parameter is referred to as the objective SCORAD (OSCORAD). In addition, the original SCORAD includes subjective elements, such as sleep disturbance and pruritus caused by eczema, which the patient rates on a 0–10 visual analog scale. In the case of EASI, the severity of erythema, infiltration, lichenification, and excoriation is assessed separately for each body region, and the extent of symptoms in each region is also determined. The final EASI score is then calculated using a specific formula. The IGA considers the presence and average severity of erythema, infiltration, lichenification, oozing, and crusting, but does not quantify the extent of the symptoms.

1.3.3 Treatment of AD

Emollient therapy is a fundamental part of basic AD treatment and should be used by all patients, regardless of disease severity, as it serves as barrier-restoring therapy. In mild disease, low-potency TCS or topical calcineurin inhibitors are typically used, whereas in moderate disease, more potent TCS are generally recommended. In moderate-to-severe eczema may require systemic treatment. During acute flares, short-term systemic corticosteroid therapy can be applied; however, long-term use is not recommended according to therapeutic guidelines. Among systemic immunosuppressive treatments, cyclosporin A is the only agent licensed for use in AD. Currently, for AD, small-molecule immunomodulatory treatments that are licensed include the JAK1/2 inhibitor baricitinib, as well as the selective JAK1 inhibitors upadacitinib and abrocitinib. Biologic therapy is also available in Hungary for patients, specifically dupilumab, an IL-4/13 receptor antagonist, which has an excellent safety profile and can be widely used in severe AD.

1.4 Allergen specific immunotherapy (AIT)

At present, AIT remains the only curative approach primarily targeting type I hypersensitivity reactions. AIT involves the controlled administration of gradually increasing doses of a specific allergen [e.g., pollen, house dust mite (HDM), bee/wasp venom, or drugs] until a maintenance dose is achieved. Depending on the severity of the allergic reaction, maintenance therapy must be continued regularly for 3–5 years, or in some cases, lifelong. Allergen delivery can be performed via subcutaneous injections or sublingual administration (SLIT), and in the case of drugs, intravenously or orally. In food allergies, AIT is currently available for peanut, egg, and milk; other food allergens are still under clinical investigation. AIT has been shown to modulate immune responses both in the short and long term in various atopic diseases. Nevertheless, the precise immunological mechanisms underlying its efficacy are not yet fully elucidated. During

therapy, pro-regulatory DCs emerge, followed within weeks by the induction of peripheral Tregs that secrete IL-10, transforming growth factor-β (TGF-β), and IL-35. Furthermore, thymus-derived FOXP3+ Treg cells are also generated. Tregs inhibit Th2 cell differentiation by producing regulatory cytokines and through direct cell-cell interactions. IL-10, in turn, promotes class-switch recombination in B cells, leading to the production of allergen-specific IgG1 and IgG4 antibodies, both associated with the establishment of immune tolerance. Allergen-specific IgG antibodies prevent the formation of IgE-allergen complexes, inhibit IgE-mediated activation of basophils and mast cells, reduce allergen presentation by B cells and DCs, and consequently suppress Th2 polarization.

Although both type I and type IV hypersensitivity mechanisms are implicated in the pathogenesis of AD, AIT may offer therapeutic benefit for symptom alleviation in selected cases. Currently, AIT is indicated in AD only in the presence of concomitant AR or allergic asthma (AA). While several studies have demonstrated improvement in AD following AIT, others have reported no significant changes in skin symptoms after treatment. However, the comparison of these studies is complicated by considerable heterogeneity in study design and patient populations. Notably, there is a lack of literature addressing how AIT may influence skin barrier function in patients with AD.

2. AIMS

In the diagnosis of the autoimmune form of CSU, currently, only expensive and technically demanding laboratory tests (such as the CD63 assay and BHRA) are available, which are typically restricted to specialized dermatology centers. This limited accessibility complicates the diagnostic process. Although current guidelines do not recommend extensive investigations due to the technical challenges associated with confirmatory testing, clarification of the autoimmune etiology can be valuable for both patients and clinicians.

One of our primary objectives was therefore to develop a simple, easily implementable combined diagnostic test with specificity and sensitivity comparable to that of the gold-standard CD63 assay.

In contrast, in AD, diagnostic testing is generally not required to establish the diagnosis. However, a straightforward diagnostic tool to monitor therapeutic efficacy could greatly assist clinicians in patient management.

Thus, another aim of our study was to investigate the previously uncharacterized effects of AIT on skin barrier function in HDM-monosensitized AD patients. In addition, we sought to assess longitudinal changes in blood immunological parameters and allergic sensitization, which were monitored in vivo by atopy patch testing (APT) and skin prick testing. To explore alterations in the skin immune microenvironment, biopsy samples were collected from APT sites for further analysis.

3. METHODS

3.1 Methods of AIU research

3.1.1 Patients of AIU research

55 patients with CSU were enrolled. Based on the contemporary guidelines, patients with a history of autoinflammatory syndromes, urticarial vasculitis, chronic inducible urticaria, hereditary angioedema, or ACE inhibitor–induced angioedema were excluded from the study. None of the patients were taking tricyclic antidepressant with antihistaminergic properties. AH treatment was discontinued at least 4 days prior to the assessments, and systemic corticosteroids or immunosuppressive agents were withdrawn at least 2 months before the study. Serum samples were collected from all participants.

Each patient completed a detailed questionnaire addressing anamnesis and clinical characteristics. All patients underwent physical examination and laboratory tests, including measurements of anti-thyroglobulin antibodies (anti-TG) and anti-thyroid peroxidase antibodies (anti-TPO). In addition, all patients were tested using the ASST and the basophil CD63 assay. A diagnosis of AIU was established based on a positive CD63 assay result.

All patients provided written informed consent prior to study participation, in accordance with the principles outlined in the Declaration of Helsinki. The study was also approved by the local ethics committee [50935/2012/EKU (776/PI/2012)].

3.1.2 Thyroid autoantibodies

The levels of thyroid autoantibodies (anti-TG and anti-TPO) autoantibodies were measured by ELISA according to the manufacturer's instructions.

3.1.3 Basophil CD63 Assay

Peripheral whole blood anticoagulated with ethylenediaminetetraacetic acid (EDTA) was obtained from atopic donors. Blood was sedimented using 6% Macrodex at 37°C for 45 minutes. The leukocyte-rich plasma was collected, washed twice with cold hydroxyethyl piperazine ethanesulfonic acid (HEPES)-EDTA buffer, and resuspended at a concentration of 10^7 cells/mL in HEPES buffer containing Ca and Mg ions. The cells were incubated with CSU patient sera for 30 minutes at 37°C. The reactions were stopped by placing the tubes on ice. Subsequently, the cells were stained with goat anti-human IgE conjugated to fluorescein isothiocyanate (IgE-FITC) and anti-human CD63 monoclonal antibody conjugated to R-phycoerythrin (CD63-PE) (Caltag Laboratories, Burlingame, CA, USA), followed by incubation for 60 minutes at 4°C. Erythrocytes were lysed using Coulter Immunoprep lysis

solution. After washing, cells were analyzed by flow cytometry using a Becton Dickinson FACScan or FACScalibur instrument. Double-positive basophils (IgE-FITC$^+$/CD63-PE$^+$) were gated, excluding cells stained only with anti-IgE-FITC. Appropriate isotype controls were used to define gating parameters. For each sample, at least 500–1000 basophils were acquired and analyzed. A result was considered positive if the percentage of CD63$^+$ basophils exceeded the 95th percentile of the response induced by sera from 20 healthy control individuals. The cutoff values were 5.1% for atopic donors and 2.2% for non-atopic donors.

3.1.4 ASST

Patient sera were separated by centrifugation at 500 g for 15 minutes at room temperature. A volume of 0.05 mL of the patient's sera was injected intradermally into the volar aspect of the forearm, choosing a lesion-free area where no wheals had been present for several hours prior to the test. As a negative control, 0.1 mL of 0.9% physiological saline was used, and a histamine solution (10 μg/mL) served as the positive control. All injections were performed approximately 4–5 cm apart. A reaction was considered positive if the diameter of the wheal induced by the patient's serum was at least 1.5 mm larger than that induced by the negative control after 30 minutes.

3.1.5 Questionnaire

All patients completed a detailed questionnaire. Using the questionnaire, we collected data on sex distribution, disease severity, and clinical course. Information regarding a personal and family history of urticaria-associated conditions, allergic diseases, and autoimmune diseases was also obtained. At the beginning of the study, the severity of CSU was assessed using the Breneman severity scoring system, as at that time the UAS7—now widely accepted—was not yet broadly implemented. In the Breneman scoring system, a four-point scale is used to evaluate the following parameters:

Localization of wheals (0 points: asymptomatic, 1 point: involvement of one region, 2 points: involvement of 2–3 regions, 3 points: generalized disease), average size of wheals (0 points: asymptomatic, 1 point: 1–2 cm, 2 points: 3–4 cm, 3 points: >4 cm), average duration of lesions (0 points: asymptomatic, 1 point: <60 minutes, 2 points: 1–4 hours, 3 points: >4 hours), frequency of wheal occurrence (0 points: asymptomatic, 1 point: twice per week, 2 points: three times per week, 3 points: more than four times per week), intensity of pruritus (0 points: none, 1 point: mild, 2 points: moderate, 3 points: severe). The intensity of pruritus was assessed by

the patients using a visual analogue scale. The final severity score was calculated by summing the individual scores; a total score above 10 was indicative of severe disease.

3.1.6 Statistics

To determine which characteristics differed significantly between patients with AIU and those without AIU, Fisher's exact test was used. Differences in age between the two groups were analyzed using the Mann–Whitney nonparametric U test ($p<0.05$ considered statistically significant). We then performed binary logistic regression to identify characteristics that independently increased the likelihood of AIU. Selected characteristics were subsequently combined with the ASST results to determine which combinations most effectively enhanced the specificity and sensitivity of ASST. For each individual parameter and combination, we calculated the specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV). All statistical analyses were performed using SPSS software, version 20.0 (IBM Corp., Armonk, NY, USA).

3.2 Methods of AD research

3.2.1 Patients of AD research

Patients with mild-to-moderate AD who were monosensitized to HDM and also suffered from AR were enrolled in the study. Based on the inclusion criteria, 14 patients were recruited (mean SCORAD: 30.1; SD: 16–50). HDM sensitization was confirmed by measuring serum allergen-specific IgE levels, as well as by performing SPT and APT.

Blood samples were collected for complete blood count and LDH level measurement. Exclusion criteria included the use of systemic immunosuppressive therapy or phototherapy within 4 weeks prior to study entry, and the use of potent topical corticosteroids within 2 weeks before baseline measurements. Biological therapies were not available at the time of the study, and thus were not considered in the exclusion criteria. Patients had no other dermatological or systemic diseases apart from AD.

Throughout the study period, all patients were permitted to use the same emollient and antihistamine, and low-potency topical corticosteroids if necessary. Topical treatments were discontinued 2 days prior to each visit to allow accurate barrier function measurements, and antihistamines were withheld 4 days prior to visits to enable in vivo skin testing.

Patients were randomly assigned to a control group (topical treatment only) or an AIT group. Eight patients (mean age 19.9 ± 9.44 years) were included in the AIT group, receiving adjuvant sublingual HDM-specific AIT in addition to topical treatment for 6 months. Patients in the AIT

group received a sublingual immunotherapy preparation according to the manufacturer's instructions, with a maintenance dose of 0.4 mL per day (equivalent to 120 IR).

The control group consisted of six patients (mean age 17.8 ± 7.17 years) who received only topical treatment for 6 months. During the study period, one patient from each group discontinued participation, and in the AIT group, one additional patient provided only blood samples at the 6-month follow-up, as skin testing could not be performed due to extensive skin lesions.

All patients provided written informed consent in accordance with the principles outlined in the Declaration of Helsinki. The study was approved by the local ethics committee [50935/2012/EKU (776/PI/2012)].

3.2.2 Clinical parameters

At both baseline and after 6 months, SCORAD and objective SCORAD scores were determined for each patient. Assessments were performed independently by two investigators, one of whom was blinded to the patients' treatment allocation. The mean of the two scores was recorded. During the same visits, patients also completed two questionnaires: the Dermatology Life Quality Index (DLQI) and the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ).

3.2.3 TEWL measurement

Transepidermal water loss (TEWL) was measured using the open-chamber device Tewameter TM300. Measurements were performed on both lesional and non-lesional AD skin areas at typical predilection sites (neck, antecubital fossa, and wrist) on both sides of the body. The assessments were conducted by an independent, blinded investigator under standardized laboratory conditions (temperature 22–25 °C, humidity 40–60%). Prior to TEWL measurement, patients were asked to rest for at least 5 minutes to allow acclimatization to the environmental conditions. Each measurement lasted 30 seconds, during which the device recorded at least 30 individual readings, and the average value was documented ($g/m^2/h$).

3.2.4 Isolation and identification of DCs

At the beginning of the study and 6 months later, we collected heparinized blood samples from all patients. Peripheral blood mononuclear cells (PBMCs) were isolated from the patients' blood samples using Ficoll Plaque Plus gradient centrifugation. CD1c+ myeloid DCs were isolated from the PBMCs using the CD1+ [BDCA1+(Blood Dendritic Cell Antigen)] Dendritic Cell Isolation Kit according to the manufacturer's instructions. The isolated and counted DCs were

then resuspended and divided into quarters in FACS (fluorescence-activated cell sorting) buffer (phosphate-buffered saline containing 1% bovine serum albumin). To identify CD1c+/CD11c+ cells, CD1c-APC (Allophycocyanin) and CD11c-FITC (Fluorescein isothiocyanate) staining were used. For cell characterization and to determine maturation and activation, the following markers were used: FcεRI-PE (Phycoerythrin), CD206-PerCP/Cy5.5 (Peridinin Chlorophyll Protein), CD83-PerCP-Cy5.5, and CD86-PE. The procedure was also performed with the appropriate negative and isotype controls (FITC Mouse IgG κ isotype control, APC Mouse IgG κ isotype control, PE Mouse IgG κ isotype control, PerCP/Cy5.5 Mouse IgG κ isotype control). Reagents were obtained from Biolegend. The CD1c, CD11c, FcεRI, CD206, CD83, and CD86 markers were incubated together for 30 minutes at 4 °C in the dark. The cells were then washed with FACS buffer and fixed with intracellular fixative buffer at room temperature for 20 minutes in the dark. After fixation, the cells were washed again with FACS buffer and kept in it until flow cytometry was performed. The expression of cell surface markers was measured by flow cytometry.

3.2.5 Identification of Treg cells

PBMCs were isolated from heparinized peripheral blood using Ficoll gradient. Intracellular Forkhead box P3 (FOXP3) staining was performed according to the manufacturer's instructions. After cell isolation, the cells were washed three times with PBS, then stained with CD4-FITC, CD25-PC5, and CD127-APC cell surface markers. The cells were then incubated in a fixative/permeabilizing buffer solution. After incubation with FOXP3-PE antibody, the cells were washed, resuspended, and measurements were performed using flow cytometry. Lymphocytes were gated based on their forward and side scatter parameters, collecting 40,000 cells. The data were analyzed using the CellQuest software.

3.2.6 Identification of Th1, Th2 and IL-10 producing Tr1 cells

Lymphocytes were stimulated with phorbol 12-myristoyl 13-acetate (PMA) and ionomycin at 37°C in a 5% CO2 environment for 4 hours. To measure de novo synthesized cytokines, the Golgi apparatus was blocked with Brefeldin-A. Cell surface CD4 was stained using quantum red-conjugated monoclonal antibodies. Following erythrolysis, the lymphocyte cell membranes became permeable, allowing intracellular cytokines to be stained with monoclonal antibodies. The cells were then fixed in paraformaldehyde. Th1 cells were stained with intracellular interferon gamma (IFN-γ), Th2 cells with intracellular IL-13, and type 1 regulatory T (Tr1) cells with IL-10.

3.2.7 Serum TSLP and IgE detection

The TSLP level in the patients' serum was determined using ELISA, namely human TSLP Quantikine Immunoassay. The amount of HDM-specific IgE was also determined using an ELISA kit according to the manufacturer's instructions.

3.2.8 Skin tests

To confirm HDM sensitization, we performed the SPT and the APT according to the manufacturer's instructions at the beginning of the study and 6 months later. In the SPT, HDM allergen extract was dropped onto the symptom-free flexor surface of the patients' forearms, and the skin was superficially scratched at the site of the drop. Histamine and physiological saline were used as positive and negative controls. The test was evaluated 15 minutes later. A test was considered positive if the positive control was positive and at least a 3mm diameter urticaria appeared at the tested site. For the APT, the allergen was placed on the lower part of the symptom-free back of the patients. The allergen was applied to the patients' skin for 48 hours in a Finnish chamber. The evaluation (a positive test was defined by erythema and infiltration) was followed by a biopsy, with the patient's consent, 48 hours after the test was applied.

3.2.9 Immunohistochemistry (IHC) analysis

Under local anesthesia, 4mm punch biopsies were taken from the patients' positive APT sites (n=8) at the beginning of the study, 48 hours following occlusion. After 6 months of SLIT, biopsies were also taken from the negative APT sites (n=5). Unfortunately, most patients did not consent to a second biopsy. As controls, we collected samples from lesional AD (n=6), non-lesional AD (n=6), and healthy dry skin (n=6) from independent volunteers. The samples were analyzed for immunologically relevant cells and mediators associated with AD.

Formalin-fixed biopsies were first deparaffinized. Epitope retrieval was performed by heat induction. The sections were stained with the following antibodies: FLG, TSLP, IFN-γ, CD4, IL-13, IL-10, IL-22, CD11C, CD83, CCL17, IgE, FOXP3, and IL-17. The stained sections were then incubated overnight at 4°C (FLG, TSLP, CD4, IL-13, CD1c, CD83, FOXP3, IL-17) or for 1 hour at room temperature (IFN-γ, IL-10, CCL17, IL-22, IgE). The secondary antibody used was horseradish peroxidase-conjugated anti-rabbit secondary antibody. Detection was performed using the Vector VIP Kit, and background staining was done with methylene green. The detection of individual proteins was performed in parallel for each section under the same

conditions to allow for comparable protein levels. Positive and negative controls were used in all cases. Each section was digitized using the Whole Slide Imaging method. A blinded examiner performed the cell counting. For each section, three regions (500µm x 500µm) were selected for cell counting. The average number of positive cells per unit area across the three regions was analyzed. To determine TSLP and FLG expression, the sections were digitized using a Zeiss plan-apochromat objective and a Hitachi 3CCD progressive scan color camera in the Pannoramic SCAN digital slide scanner. Immunostaining analysis was performed using Pannoramic Viewer 1.15.2 software with HistoQuant application. Three regions of interest (ROI) were designated for each section. The software settings then determined the field area (FA) and mask area (MA). The FA represents the entire area of the ROI, while the MA represents only the positive areas. The MA/FA ratio was determined for each ROI. The absolute TSLP and FLG expression levels were calculated by dividing the MA by the FA and multiplying by the epidermal length for each sample.

3.2.10 Statistics

The statistical analysis was performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). All datasets were subjected to the Kolmogorov-Smirnov test for normality. For comparisons of IHC, clinical, and skin barrier parameters between groups, ANOVA followed by Tukey post hoc test was used (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). The changes in the laboratory test results were determined, and the comparison of changes was performed using a two-sample t-test.

4. RESULTS

4.1 Results of AIU research

4.1.1 Differences in clinical and laboratory parameters between $CD63^+$ AIU and $CD63^-$ non-AIU patients

A total of 55 patients (19 males and 36 females) were enrolled in the study, with a mean age of 49.2 years (SD = 15.15) and a mean disease duration of 18.4 months (SD = 14.5). The diagnosis of AIU was established based on basophil CD63 assay positivity. In total, 33 patients (60%) were $CD63^+$ and diagnosed with AIU, while 22 patients (40%) were $CD63^-$ and therefore classified as having non-AIU CSU.

Subsequently, all patients (both AIU and non-AIU) underwent ASST, Breneman scoring, laboratory examinations, and detailed collection of clinical and anamnestic data.

ASST positivity (87.9% in AIU vs. 22.7% in non-AIU), the presence of nocturnal symptoms (81.8% vs. 45.5%), angioedema (81.8% vs. 27.3%), symptoms occurring on more than five days per week (97% vs. 40.9%), and the presence of thyroid autoantibodies (63.6% vs. 9.1%) were all significantly more frequent among AIU patients.

Although the following parameters did not reach statistical significance, they were more commonly observed in the AIU group: female predominance (81.8% vs. 72.7%), duration of a single wheal lasting 5–24 hours (84.8% vs. 68.2%), and the need for higher-dose antihistamine therapy (72.7% vs. 54.5%). Thyroid disease was reported with a similar frequency in the medical history of both groups (18.2% in AIU vs. 13.6% in non-AIU), as was the proportion of patients with severe urticaria, defined by a Breneman score >10 (21.2% vs. 22.7%).

Vitiligo and the use of other therapeutic modalities such as cyclosporine, plasmapheresis, or intravenous immunoglobulin were observed exclusively in the AIU group; therefore, p-values could not be calculated for these parameters.

4.1.2 Odds ratio, specificity, and sensitivity of parameters significantly associated with the diagnosis of AIU

The binary logistic regression method was used to determine the odds ratios (OR) of individual parameters in order to identify factors increasing the likelihood of AIU. The odds ratio for ASST positivity was 24.65 (95% confidence interval: 5.81–104.53). All parameters that were significantly more frequent among AIU patients — namely ASST positivity, the presence of nocturnal symptoms, angioedema, symptoms occurring on more than five days per week, and thyroid autoantibody positivity — significantly increased the odds of AIU.

The sensitivity and specificity of the ASST were 88% and 77%, respectively. The positive predictive value (PPV) was 85.2%, and the negative predictive value (NPV) was 81%. None of the other significantly more frequent parameters exceeded the sensitivity, specificity, PPV, or NPV values of the ASST.

4.1.3 Specificity and sensitivity of the combined test including ASST, clinical, and laboratory parameters

Using logistic regression analysis, we evaluated all possible combinations of parameters that occurred significantly more frequently among AIU patients in order to identify the combination with the highest sensitivity and specificity. With this method, we first examined how the sensitivity and specificity of the ASST changed when individual parameters were added. When ASST was combined with the presence of nocturnal symptoms or angioedema, neither the sensitivity nor the specificity of the skin test changed. When ASST was combined with the presence of symptoms on more than five days per week, sensitivity remained 88%, while specificity increased to 91%. When ASST was combined with thyroid autoantibody positivity, sensitivity increased to 91%, whereas specificity decreased to 68%. These results indicate that combining ASST with a single additional parameter did not substantially improve both sensitivity and specificity.

When ASST was combined with two anamnestic or clinical parameters, the best-performing combination included the presence of symptoms on more than five days per week and angioedema, resulting in a sensitivity of 97% and specificity of 82%. When ASST was combined with these two characteristics and thyroid autoantibody positivity, sensitivity reached 94% and specificity increased to 86%. The highest sensitivity (97%) and specificity (86%) were achieved when ASST was combined with all parameters that were significantly more frequent among AIU patients.

4.2 Results of AD research

4.2.1 Clinical and barrier results

Clinical response was assessed using SCORAD, OSCORAD, DLQI, and RQLQ, while skin barrier function was evaluated by measuring TEWL in both the control and AIT groups at baseline and after 6 months. Baseline values did not differ between the two groups. After 6 months, all clinical parameters except DLQI improved significantly in the AIT group, whereas no significant changes were observed in the control group during the observation period.

At baseline, TEWL measured on lesional and non-lesional skin did not differ significantly between the two groups. However, within each group, TEWL values on lesional and non-lesional skin were significantly different at baseline. After 6 months, barrier function values remained unchanged in the control group, whereas in the AIT group, TEWL on both lesional and non-lesional skin improved significantly.

4.2.2 Immune laboratory results

Peripheral blood was analyzed to assess parameters of DC precursors, including dermal myeloid DCs and inflammatory epidermal dendritic cells, as well as inflammatory and regulatory T-cell subsets (Treg, Tr1, Th1, Th2). In addition, type I hypersensitivity-related markers (serum total IgE, eosinophil count) and AD-associated markers (TSLP, LDH) were evaluated at baseline and at 6 months in both groups. For DC analysis, $CD1c^+/CD11c^+$ pre-DCs were gated (over 90% of gated cells were positive for both CD1c and CD11c), and FcεRI expression and cell surface markers (CD206, CD83, CD86) were measured. At baseline, no significant differences were observed between the groups in the expression of any of these molecules. Similarly, at 6 months, no significant differences were detected for any marker, although a non-significant decrease in CD83 and CD86 expression was observed in the AIT group.

Throughout the study, no significant differences were noted between the two groups in the proportions of $CD4^+$ T-cell subsets (Treg, Tr1, Th1, Th2, Th1/Th2 ratio) or in serological immune markers (TSLP, total IgE, LDH, eosinophil counts) at baseline or after 6 months.

4.2.3 Results of skin prick tests and atopy patch tests

At baseline, all patients (n = 14) underwent APT with HDM allergen on intact, non-lesional skin. A positive APT was an inclusion criterion, as it confirmed the presence of cutaneous sensitization. After 6 months, APT was repeated in all patients. In the control group, APT remained positive in all patients, whereas in the AIT group, APT became negative in all patients (with one patient not eligible for testing). At both baseline and 6 months, SPT with HDM allergen was also performed. In all cases, including both the control and AIT groups, SPT remained positive throughout the study period.

4.2.4 Examination of FLG, DC, T cell infiltration by IHC in APT samples

Biopsy samples were collected from patients with positive (n = 8) and negative (n = 5) APT results to examine immunological and barrier characteristics. As controls, lesional and non-lesional AD skin samples, as well as skin from healthy subjects, were included.

Prior to AIT, allergen exposure induced prominent DC infiltration and mixed T-cell infiltrates in the skin of positive APT samples. Infiltrating T cells included IFN-γ^+, IL-13^+, IL-22^+, IL-17^+, IL-10^+, and FoxP3^+ subsets. Compared with other sample groups, the infiltration pattern of positive APT samples most closely resembled that observed in lesional AD skin.

In contrast, negative APT samples exhibited a marked reduction in DC and CD4^+ T-cell infiltration. Although decreases were also observed in DC markers CD83 and CCL17, as well as in IFN-γ^+, IL-13^+, IL-22^+, IL-17^+, IL-10^+, and FoxP3^+ T cells, these differences were not statistically significant. The infiltration pattern in negative APT samples resembled that of non-lesional AD skin.

Skin IgE expression was high in positive APT samples and decreased in negative APT samples, although the difference was not statistically significant. FLG expression increased significantly in negative APT samples compared with positive APT samples, indicating improved barrier function following AIT. Epidermal TSLP levels did not differ significantly between positive and negative APT samples.

Overall, our findings indicate that the barrier and immune composition of positive APT samples resembled lesional AD skin, whereas the immune status of negative APT samples following AIT was similar to that of non-lesional AD skin.

5. DISCUSSION

AIU and AD are common, often extensive, inflammatory immune-mediated, non-allergic disorders that frequently present with severe symptoms and comorbidities. The diagnosis of specific subgroups of these diseases, as well as the broad application of diagnostic tests, can pose significant challenges even for experienced clinicians.

Challenges arise not only in the diagnosis of AIU but also in defining the disease itself, as its definition evolves with an improved understanding of its underlying mechanisms. The condition previously referred to as chronic idiopathic urticaria has only been distinguished as AIU in the literature since 2013. At the time of our investigations, AIU was defined as a chronic, non-inducible urticaria characterized by the concurrent positivity of both ASST and BHRA or CD63 assays. It was already known that in AIU, autoantibodies are produced against both IgE and FcεRI; moreover, IgE-type autoantibodies can bind directly to FcεRI, thereby activating it. The current literature distinguishes these two types of AIU: autoreactive and autoallergic CSU. However, the two groups do not separate clearly on a clinical basis, as approximately half of patients may exhibit features characteristic of both autoreactive and autoallergic AIU. With the growing understanding of the underlying pathomechanisms, previous and more recent studies are often difficult to compare, since definitions and diagnostic criteria vary.

The diagnostic screening method for AIU is the ASST. This is a rapid and simple test that can be relatively easily performed in outpatient settings. A diagnostic test is considered appropriate if it demonstrates high specificity and sensitivity, along with adequate PPV and NPV. One of the objectives of our study was to improve the diagnostic performance of the ASST by retaining the original test while combining it with clinical and laboratory data. We hypothesized that this approach could enhance the test's specificity and sensitivity without requiring costly equipment, thereby allowing the diagnosis to be established without referring patients to larger specialized centers. We selected laboratory tests that are widely available, inexpensive, and recognized in the literature as potential biomarkers for AIU. Combinations of these parameters were evaluated to increase the sensitivity and specificity of the ASST. The optimal combination included ASST positivity, the presence of symptoms on more than five days per week, occurrence of angioedema, nocturnal symptoms, and thyroid autoantibody positivity. Using this combination, ASST sensitivity increased to 97% and specificity to 86%. These values correlated well with the sensitivity (95.5%) and specificity (90.5%) of the basophil CD63 assay. Although the guidelines in effect at the time of our study recommended only limited routine diagnostic measures for CSU and suggested ASST only as an extended diagnostic step, we

believe that identifying the AIU subgroup is essential. Our experience indicates that AIU patients exhibit greater therapeutic resistance than non-AIU patients, which negatively impacts their quality of life. It would be worthwhile to incorporate the inexpensive and simple ASST into routine assessments, combined with thyroid autoantibody measurement and clinical data, to facilitate AIU diagnosis. Such a combined test, characterized by high sensitivity and specificity, can simplify the diagnosis of AIU and be readily applied in an outpatient setting.

In contrast to AIU, establishing a diagnosis of AD is straightforward in most cases, as it is based on clinical presentation according to the criteria developed by Hanifin and Rajka. Similar to AIU, there is currently no reliable biomarker to monitor therapeutic response in AD. For both conditions, the effectiveness of a therapy can be assessed at a given time point through physical examination, but there is no test or laboratory parameter available that can predict which therapy will be most effective for an individual patient.

Prior to our study, there was no evidence that AIT could modify skin barrier function or allergen-specific sensitization in the skin of AD patients. Considering the available literature, we were the first to investigate how adjuvant AIT affects the physico-chemical characteristics of the skin barrier. Improvements in TEWL were observed only in the AIT group and were significant in both lesional and non-lesional AD skin. Enhancement of skin barrier function paralleled clinical improvement and was observed exclusively following adjuvant AIT. The improvement in barrier permeability was further supported by a significant increase in epidermal FLG expression, as demonstrated by IHC in the APT-negative skin of patients in the AIT group.

To date, the literature has not described how APT results change following AIT. In our study, we observed that after adjuvant AIT, all patients who had previously tested positive on APT became clinically negative, indicating that the desensitizing effects of AIT were detectable in alterations of the cutaneous immune system. In contrast, SPT responses remained positive in all patients at all time points. This finding aligns with the observation that serum IgE levels did not change and that IgE expression in the skin decreased only slightly following AIT. These results are consistent with previous reports indicating that SPT is not suitable for assessing the efficacy of AIT.

Given that APT results correlated with both clinical and barrier responses to AIT, they played a central role in our investigations. We hypothesized that changes in the immunological and barrier environment of APT sites could serve as histological footprints of the beneficial effects of adjuvant AIT. IHC analyses were performed to examine cellular and cytokine/chemokine environment changes before and after immunotherapy in APT-positive and -negative skin. IHC

revealed a marked reduction in dendritic cells and T cells in APT-negative skin following AIT, compared with APT-positive skin. No significant alterations were observed in T-cell subset composition within the skin over the course of the study. Only a mild, non-significant decrease in skin IgE expression was noted, which may explain the persistence of positive SPT results after AIT. Furthermore, a significant increase in FLG expression was observed in APT-negative skin after AIT, in contrast to the reduced FLG expression in APT-positive skin, indicating improved barrier function. Histologically, negative APT skin closely resembled non-lesional AD skin. Our study clearly demonstrated that adjuvant AIT in HDM-sensitized AD patients, who also suffer from concomitant allergic rhinitis, led to rapid clinical and barrier improvements and a marked reduction in cutaneous sensitization. In line with current therapeutic guidelines, our findings suggest that AIT may serve as a useful adjunctive treatment for appropriately selected AD patients (specifically those with concomitant allergic rhinitis), and that APT may be a valuable biomarker for assessing and monitoring the efficacy of immunotherapy.

6. SUMMARY

Biomarkers, supportive to the diagnosis of certain conditions or the monitoring of therapeutic efficacy in a cost-effective manner, were studied in common immunological skin diseases.

In autoimmune urticaria (AIU), we developed a combined diagnostic test that significantly improved sensitivity (from 70% to 97%) and specificity (from 80% to 86%) of the autologous serum skin test. These metrics are comparable to those of *in vitro* gold standard tests (such as basophil histamine release assay and CD63 basophil activation test), which are costly. We identified specific clinical history and laboratory parameters characteristic of AIU and combined them into a diagnostic model. The final combined test includes one laboratory marker (elevated anti-thyroid peroxidase level) and several clinical features (presence of night-time symptoms, symptom-duration longer than five days in a week, and angioedema).

In atopic dermatitis (AD), some patients develop sensitization to various allergens, most commonly to house dust mites (HDM), which can worsen skin symptoms. Allergen-specific immunotherapy (AIT) offers a causal treatment for established allergies. In our study, AD patients with HDM monosensitization and concurrent allergic rhinitis were treated with AIT. This was the first study to examine the effect of AIT on skin barrier function. Both clinical and skin barrier parameters improved following AIT. The atopy patch test (APT) turned negative in all patients receiving AIT, clearly indicating the effectiveness.

We also performed skin biopsies from the APT sites, and immunohistochemical analysis showed a decrease in the inflammatory cytokine milieu characteristic of AD after AIT, although it did not return to levels seen in healthy individuals. Additionally, an improvement in skin barrier integrity was also observed in the biopsy samples.

7. NEW FINDINGS

In the present study, we were the first to develop a combined diagnostic test supporting the identification of AIU, which provides sufficiently high specificity (86%) and sensitivity (97%) for accurate disease recognition. The diagnostic panel comprises three routinely applicable components: the ASST, the assessment of thyroid-specific autoantibodies, and the evaluation of selected clinical–anamnestic features (occurrence of nocturnal wheals, symptoms present on more than five days per week, and the presence of angioedema). Our results indicate that this combined test is suitable for the high-accuracy identification of AIU and may support clinical decision-making.

In our research, we were also the first to investigate the effect of AIT on skin barrier function. AIT resulted in significant improvement both in clinical parameters and in indicators of skin barrier integrity. Treatment-related changes could also be monitored using the APT: in all patients receiving AIT, the APT became negative throughout the course of therapy. Immunohistochemical analysis of the negative APT samples confirmed that the method sensitively reflects AIT-induced improvements in barrier function as well as the attenuation of cutaneous inflammation.

Although AIT reduced the inflammatory activity of the skin, inflammation did not fully resolve in any of the examined cases. The post-treatment skin phenotype resembled the status typically seen in asymptomatic patients with AD. This observation suggests that AIT does not eliminate the underlying disease itself, which is consistent with the understanding that AD is not primarily an allergic disorder. Nevertheless, our findings demonstrate that when symptoms are driven by an identifiable allergen, AIT can produce clinically and histologically verifiable improvements in skin barrier function and reduce cutaneous inflammation.

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Total IF of journals (all publications): 67,032
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