Biomarkers for phenotype-endotype relationship in atopic dermatitis: a critical review

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Summary

Atopic dermatitis (AD) is the most common form of chronic skin inflammation with diverse clinical variants. Historically, various AD phenotypes have been grouped together without considering their heterogeneity. This approach has resulted in a lack of phenotype- and endotype-adapted therapeutic strategies. Comprehensive insights into AD pathogenesis have enabled precise medicinal approach for AD. These efforts aimed to redefine the endophenotype of AD and develop various biomarkers for diverse purposes. Among these endeavours, efforts are underway to elucidate the mechanisms (and related biomarkers) that lead to the emergence and progression of atopic diseases originating from AD (e.g., atopic march). This review focuses on diverse AD phenotypes and calls for a definition of endophenotypes. While awaiting scientific validation, these biomarkers ensure predicting disease onset and trajectory and tailoring therapeutic strategies for the future.

Keywords: Atopic dermatitis; Atopic march; Biomarker; Endo-phenotype; Precision medicine

Introduction

Atopic dermatitis (AD), the most common chronic inflammatory skin disease, is characterized by intense itching sensation with varying degrees of cutaneous inflammation. Recent studies on the barrier function and immunology of AD have significantly deepened our insight into AD pathogenesis. Moreover, genetic and epidemiological research has revealed new dimensions regarding the persistence and natural history of AD, including atopic march (AM). Those recent research has spurred the development of new treatments targeting Th2 cytokines and chemokines in AD.[1]

For instance, dupilumab was approved by the FDA in 2016 and the EMA in 2017, followed by the EMA's approval of baricitinib in 2020 and tralokinumab in 2021. Despite the development of revolutionary new therapeutics, research on AD endotypes and phenotypes for clinical application is just beginning. Various studies have explored how age, geographical background, IgE sensitivity, and allergic comorbidities affect AD's clinical phenotype, alongside molecular profiling through serum or skin tissue to categorise AD endotypes.[2,3] Based on those studies, efforts to classify patients by clinical features or biomarkers are ongoing, with their practical clinical utility still being investigated.

Although high clinical heterogeneity of AD is well recognized by clinicians and researchers, most AD treatments follow a one-size-fits-all approach. The development of AD therapies targeting specific phenotypes and endotypes is overlooked. However, precision medicine, using endo-phenotypic criteria for patient stratification, is expected to replace traditional treatments. Identifying reliable biomarkers for tailored treatments is vital, promising advances in AD management, such as prevention, choosing effective treatments, and timing medication changes.

In this review, we aim to summarize the current understanding of the endotypes and phenotypes of AD through a thorough literature review. Also, we intend to outline the status of biomarkers being developed/used for various purposes such as screening, diagnosis, severity assessment, prognosis prediction, and monitoring of AD. Additionally, we plan to compile information on biomarkers that could enable a precision medicine approach to AD.

The urgent need for the development of biomarkers that reflect disease endotypes

AD features a complex clinical phenotype with diverse symptoms and trajectories.[1] This heterogeneity results in mixed responses to conventional treatments, risking adverse effects from high therapeutic doses in patients with poor clinical response. Precision medicine aims to give the right drug in the correct dosage to the appropriate patient at the optimal time, with biomarkers playing a key role.

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AD management often uses a 'one-size-fits-all' strategy, mainly based on physician assessment and disease severity. In current clinical settings, severity scoring systems such as Eczema Area and Severity Index (EASI) and SCORing Atopic Dermatitis (SCORAD) are widely utilised. However, these tools do not reflect the detailed endophenotypes, and there exists potential for subjective interpretation by the physician. Hence, there's a pressing need for new biomarkers to accurately categorise AD endo-phenotypes. Additionally, recent biologics and small molecules typically depends on the severity level of AD. However, recognizing AD's evolving endophenotypes could enable more accurate subgroup identification, improving therapy response predictions, risk-benefit analysis, and minimizing side effects for those unlikely to benefit from certain treatments. This approach underlines the necessity for precision-medicine concept in AD management, highlighting the role of advanced biomarkers and patient-specific treatment strategies.

New biomarkers can be linked to 'disease modification' concepts, highlighting the importance of targeting the underlying processes of diseases to alter their course.[4] Therapeutics in AD focusing on core immune pathways (Th2 response) are growing, yet there is still an unmet need. For instance, global trials show dupilumab achieves EASI-75 and EASI-90 in 44–65% and 30–51%, respectively,[5] leaving some patients unresponsive, underscoring the need for treatments tailored to varied endo-phenotypic profiles to close the treatment gap. Furthermore, the European Medicine Agency (EMA) defines 'disease modification' as effects monitored through multiple validated biomarkers reflecting pathophysiological disease progression. Thus, development of new drugs should proceed alongside efforts to decipher the disease's pathophysiological mechanisms by endophenotypes and subsequent development of validated biomarkers.

Endeavours to develop new therapeutics for AD have focused on alleviating symptoms and preventing flare-ups, yet they often overlook associated atopic comorbidities like AM, which includes allergic asthma, rhinitis, and food allergies. Treating these as separate entities can be beneficial for creating disease entity-specific therapeutics, but may ignore the disease-modification concept of AD. Despite several cohort studies,[6] there is still lack clinical and laboratory biomarkers to identify those at high risk of AM.[1] Not all patients with early onset AD progress through AM, but the challenge is to identify those at the highest risk and developing personalized therapeutic/preventive strategies. To fully understand the allergy pathway from the skin to the upper/lower airway mucosa, a comprehensive understanding of paediatric AD endotypes and phenotypes, skin barrier dysfunction, and innate/adaptive immune processes involved in the protection of barrier surfaces from inflammation, allergen sensitization, is required.[7]

From physiopathology to phenotypes: multiple aspects of AD endophenotypes

Most of our understanding of AD pathogenesis originates from research on the adult skin. As children are not miniature adults, the pathogenesis of adult AD cannot be assumed to be identical. T cell and dendritic cell infiltrates, with Th2/Th22 skewing and high expression of interleukin (IL)-31 in the skin versus control skin, are also found in infants and toddlers with new-onset AD (onset within the last six months).[8] However, only CLA+ CD4+ Th2 cells have been detected in the blood of infants and toddlers.[9] These children do not have CLA- Th2, CD8+, or Th22 cells, present in adult AD blood.[10] Furthermore, early onset paediatric AD skin samples lack the Th1 response, which is a characteristic of adult AD; instead, Th9 and Th17 responses are greatly upregulated.[8,11] In addition, epidermal cell hyperproliferation is greater in young children than that in chronic adult AD, as shown by the high expression levels of keratinocyte genes encoding keratin16 and Ki67.[12] Additionally, the expression of epidermal differentiation and cornification products is relatively common in paediatric AD,[9,11] whereas it is downregulated in adults with AD. In young children, both lesional and non-lesional skin demonstrated elevated filaggrin expression. This is especially intriguing because despite having normal filaggrin levels, these young children have extremely high transepidermal water loss, implying that other components of the barriers are defective in early onset childhood AD.[9] These distinctions between paediatric and adult AD highlight the varied phenotypes across ages and suggest the need for developing age-specific biomarkers for AD.

Recent studies have discovered that different ethnic backgrounds display distinct clinical and molecular phenotypes.[13] The transcriptomic profiles of European descent differ from those of Asian descent with AD.[14] Asian AD phenotypes present as a blended phenotype between AD and psoriasis, including increased epidermal hyperplasia, parakeratosis, and higher Th17 activation.[15] Furthermore, skin lesions in Japanese and Korean AD patients have been found to produce potent Th17 cells. Th17-driven skin inflammation represents more clinically pronounced lichenification with profound epidermal hyperplasia in Asian patients with AD. These findings show that based on the geographical diversity, there may be different clinical phenotypes,[14] which indicates the necessity of establishing biomarkers according to patient ethnicity.

The earlier onset of AD compared to asthma and allergic rhinitis indicates a causal association between the development of other atopic diseases and the

prevalence of AD. In general, AD precedes the onset of food allergy, asthma, and allergic rhinitis, implying that AD may be a "gateway" for later atopic comorbidities. This concept is supported by studies, such as those in mice where skin sensitisation led to airway inflammation without direct airway exposure, highlighting the skin's role in IgE sensitisation. Despite the lack of a universally accepted definition, the current concept of AM implies the onset of AD, followed by an increased risk of developing one or more atopic comorbidities characterised by IgE-mediated allergies. Thus, AM underscores the importance of early AD intervention to prevent the development of allergic comorbidities.

Despite variations in methodology among epidemiological studies, recent research using machine learning has identified similar patient clusters or endotypes.[16] The most recent epidemiologic studies include the Canadian Healthy Infant Longitudinal Development birth cohort and Protection Against Allergy: Study in Rural Environments (PASTURE) studies, which indicated that presence of food allergy and a persistent AD phenotype are risk factors for the progression of airway type 2 responses.[17] Efforts to merge diverse cohorts have reinforced the results of previous cohort studies. Paternoster et al. combined the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Prevention and Incidence of Asthma and Mite Allergy cohorts to include 13,546 children across the United Kingdom and the Netherlands.[6] In this study, the persistent phenotype had the highest probability of developing coexisting asthma, elevated IgE levels, and a family history of atopy. Belgrave et al.'s study, combining ALSPAC and Manchester Asthma and Allergy Study cohorts of 9,801 children and employing Bayesian machine learning, validated AD persistence and sensitisation status as major AM risk factors.[18]

Current candidate biomarkers of AD

While advancements in defining traditional clinical phenotypes of AD have been made, the exploration of endophenotypes through biomarker discovery is just beginning. The process of assessing and validating various biomarkers for clinical use represents a significant step towards their incorporation into real clinical fields. Notably, some biomarkers, like TARC, are already in use in clinical settings, highlighting their potential immediate impact on patient care (Table 1, Fig. 1). While the most of biomarkers discussed in the subsequent section have yet to demonstrate efficacy in real-world clinical settings, it's crucial to appreciate the context of their ongoing development. These biomarkers offer the prospect of identifying new endophenotypes and leading the way in innovative treatment approaches, potentially transforming the clinical management of AD.

Screening biomarkers

Screening biomarkers for early childhood AD can identify those at risk, offering a proactive approach to management. Studies suggest that early interventions, like applying moisturisers to newborns with a family history of AD, could delay its onset, though evidence varies.[61] Using screening biomarkers to pinpoint high-risk individuals is gaining acceptance for its potential to predict AD, break its chronic cycle, and prevent AM. Identifying effective biomarkers or their combinations could enable early interventions. Recently, measuring transepidermal water loss has emerged as a promising non-invasive biomarker for this purpose.[62]

Genome-wide studies have linked several genes, notably filaggrin, to AD development, with about 30% of patients showing filaggrin mutations.[20] Screening for filaggrin and other skin protein gene mutations could identify those at high risk for AD or related allergies like AM.[21] Additionally, Toll-like receptor 2 (TLR2) polymorphism (TLR2 16394A > T) is associated with AD and other allergic comorbidities.[25] Currently, genotyping is costly and time-consuming, but advancements like next-generation sequencing could soon make gene profiling accessible for AD screening.

The serine protease inhibitor Kazal-type 5 (SPINK5) and thymic stromal lymphopoietin (TSLP) are considered to be potential screening biomarkers for AD, similar to filaggrin-like epidermal proteins.[23,26] Furthermore, elevated cord blood IgE levels during pregnancy, lymphotoxin-alpha genotype alteration, and FcεR1-beta genotype alteration[24,47] have all been associated with childhood AD, indicating that they may be used as screening biomarkers for the disease.

Diagnostic biomarkers

Since AD is primarily diagnosed based on clinical and morphological characteristics, no biomarker currently validates its diagnosis. The standardised clinical features of AD in infancy, childhood, and adolescence have been used as diagnostic criteria. However, infants under three months of age and senile AD are difficult to diagnose as their clinical features vary from those of classical AD. Although research on diagnostic biomarkers for both subtypes is still underway, no candidates have emerged.

Biomarkers for differential diagnosis of AD have been developed instead of definitive diagnostic biomarkers. The carbonic anhydrase II (CA II) gene is highly expressed in eczematous diseases, which can aid in the exclusion of mimicry of AD, such as psoriasis.[57,58] In addition, neuron-specific Nel-like protein 2 (NELL 2) is reported to be highly expressed in the AD epidermis,[58] which reflects the hallmark symptom of AD, pruritus. In addition to this new molecular entity, sampling routes other than those through the skin or blood have also been investigated. Analysis of the urinary lipid

Biomarkers	Function	Category of biomarkers	References
Genotypes			
Filaggrin (FLG) genotypes	Provides structural and mechanical integrity to skin, FLG degradation products account in part for the water-holding capacity and maintenance of acidic pH of the SC	Screening biomarker, Preventive biomarker	20–22
SPINK5 genotypes	Codes for Lympho-epithelial Kazal-type-related inhibitor (LEKT1). Regulation of desquamation via its ability to selectively inhibit KLK5, KLK7, and KLK14	Screening biomarker	23
FcεRI-β genotypes	High-affinity receptor for the Fc region of immunoglobulin E	Screening biomarker	24
Toll-like receptor 2 (TLR 2)	Recognizes a variety of microbial components derived from Gram-positive bacteria, such as lipopeptides, peptidoglycan, and lipoteichoic acids	Screening biomarker	25
Lymphotoxin alpha (LT-α)	Produced by lymphocytes and significantly contributes to the proliferation of B-cells and the synthesis of IgE.	Screening biomarker	24
TSLP	Secreted by epithelial cells in the skin, directly interacts with T cells to promote a Th2 response	Screening biomarker	26
Chemokines			
TARC/CCL17	Attracts CC chemokine receptor 4-positive (CCR4+) or CCR8+ cells	Severity biomarker, Monitoring biomarker	22,27–30
MDC/CCL22	Attracts CC chemokine receptor 4-positive (CCR4+) cells	Severity biomarker, Monitoring biomarker	22,28
CTACK/CCL27	Attracts CC chemokine receptor 10-positive (CCR10+) cells	Severity biomarker, Monitoring biomarker	22,28,31
PARC/CCL18	Attracts CC chemokine receptor 8-positive (CCR8+) cells	Severity biomarker, Monitoring biomarker	29,32
Eotaxin/CCL26	Attracts CC chemokine receptor 3-positive (CCR3+) cells	Severity biomarker	33
Interleukins			
IL-22	Main driver of epidermal hyperplasia and barrier defects Promotes keratinocyte proliferation Inhibits keratinocyte terminal differentiation	Severity biomarker, Monitoring biomarker, Endotypic biomarker	29,34–37
IL-13	Key Th2 cytokine that drives inflammation in the periphery	Severity biomarker, Endotypic biomarker	9,38,39
IL-18	Pro-inflammatory cytokine that exerts pleiotropic effect that stimulates both Th2 and Th1 responses	Severity biomarker, Monitoring biomarker	40
IL-19	Pro-inflammatory cytokine that probably stimulates the production of Th2 cells	Severity biomarker	9,41
IL-31	Main driver of chronic pruritus in atopic dermatitis	Severity biomarker, monitoring biomarker (pruritus)	42,43
IL-33	Main driver of epithelial alarmin systems Drives robust Th2 responses	Severity biomarker	31
IL-16	Induces chemotactic responses in CD4+ T cells, monocytes, and eosinophils	Severity biomarker	44
Others			
Periostin	Supports adhesion and migration of epithelial cells	Severity biomarker, Endotypic biomarker	39,45,46
DPP-4	Induced by IL-4 and IL-13, is involved in T-cell activation and regulation	Endotypic biomarker	39
Serum total IgE	Binds allergens to degranulate mast cells in various allergic diseases	Severity biomarker	28
Cord blood IgE	Binds allergens to degranulate mast cells in various allergic diseases	Screening biomarker	24,47
Malassezia-specific IgE	Specific IgE to *Malassezia* fungi. It denotes an immunological hypersensitivity to *Malassezia* species	Severity biomarker, Endotypic biomarker	48,49
Eosinophil counts, ECP	Releases eosinophil basic proteins upon activation via Th2 cytokines	Monitoring biomarker	50,51
Soluble IL-2R	Soluble cytokine receptor which secreted upon T-cell activation. Elevated serum levels are observed across a range of immune-related conditions	Monitoring biomarker	29,30
S100A7/A8/A9//A12	Anti-microbial peptides; preferentially kills *E. coli*	Severity biomarker,	9,38,52,53
E-selectin	Allows the adhesion of neutrophils, monocytes, and leukocytes on stimulated endothelium in the skin	Monitoring biomarker	10,28
RAB25	A member of the RAB11 small GTPase subfamily, which orchestrates intracellular vesicle trafficking	Severity biomarker	54
MMP8, 9, 12	Protease involved in macrophage migration, could also have a crucial role in regulating the resolution of inflammation	Severity biomarker	10,38,41,55
LDH	Tetrameric oxidoreductase enzyme; non-specific marker of tissue turnover	Severity biomarker, Monitoring biomarker	28,56
CA II	Carbonic anhydrase catalyses the reversible hydration of carbon dioxide	Diagnostic biomarker	57,58
NELL2	Important for neuronal polarization and axon growth	Diagnostic biomarker	58
EDN	Secretory proteins of eosinophils, it has broad antiviral activity, targets RNA viruses	Severity biomarker	50
SCCA2	Member of the ovalbumin serpin (ov-serpin)/clade B serpin family	Severity biomarker	33,59

(Table 1 continues on next page)

Biomarkers	Function	Category of biomarkers	References
(Continued from previous page)			
Urine PGF_{2a}, PGE_2, PGD_2	Pivotal mediators in the pathogenesis of allergic inflammation, modulating vascular permeability, bronchial smooth muscle tone, and leukocyte recruitment,	Diagnostic biomarker	60

FLG, filaggrin; SPINK5, serine protease inhibitor Kazal-type 5; TARC, thymus and activation-regulated chemokine; MDC, macrophage-derived chemokine; CTACK, cutaneous T-cell-attracting chemokine; PARC, pulmonary and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; ECP, eosinophil cationic protein; MMP, matrix metalloproteinase; LDH, lactate dehydrogenase; CA II, Carbonic anhydrase II; NELL 2, Nel-like protein 2; EDN, Eosinophil-derived neurotoxin; SCCA2, Squamous Cell Carcinoma Antigen 2; DPP-4, Dipeptidyl Peptidase 4.

Table 1: **Current candidate biomarkers in AD.**[19]

profile using liquid chromatography-tandem mass spectrometry showed that prostaglandins, such as PGF_{2a}, PGE_2, PGD_2, and arachidonic acid metabolites, were increased in the urine of patients with AD.[60]

Severity biomarkers

Most published biomarkers have been used to determine the severity of AD (i.e., severity biomarkers). Thymus and activation-regulated chemokine (TARC),[22,27–30] macrophage-derived chemokine (MDC),[22,28] cutaneous T cell-attracting chemokines (CTACK),[22,28,31] Eotaxin/CCL26,[33] IL-13,[9,38,39] IL-31,[42,43] IL-33,[31] IL-22,[29,34–37] IL-18,[40] IL-19,[9,41] IL-16,[44] pulmonary and activation-regulated chemokine (PARC),[29,32] periostin,[39,45,46] S100A7/8/9/12,[9,38,52,53] MMP8/9/12[10,38,41,55] and lactate dehydrogenase (LDH)[56] are among the most highly rated severity biomarkers. Additionally, serum eosinophil-derived neurotoxin (EDN)[50] and serum squamous cell carcinoma antigen 2 (SCCA 2)[33,59] are reported to be positively correlated with the severity of AD. RAB25, a promising severity biomarker, represents epidermal barrier function impairment in AD.[54] Recent study have shown that RAB25 expression in the skin is significantly lower in patients with AD. Moreover, RAB25 expression negatively correlated with the EASI score. Along with

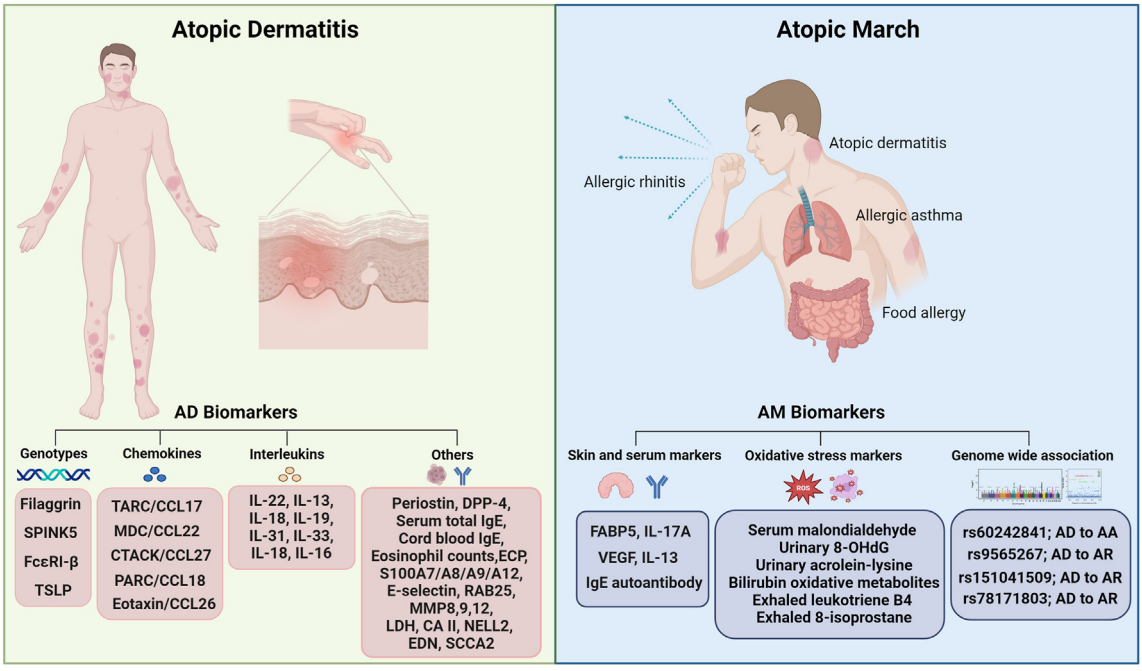

Fig. 1: Potential biomarkers for AD and AM. The left panel represents the current candidates of biomarkers for AD. Genotypes of the specific genes, chemokines and cytokines which reflect the Th2 immune responses, innate immune responses, and other biomarkers could be a potential biomarkers of AD. The right panel represents the current candidates of biomarkers for AM. Skin and serum biomarkers include FABP5, IL-17A, VEGF, and IL-13. Oxidative stress-related biomarkers also could be used as biomarkers for AM. (SPINK5; Serine peptidase inhibitor Kazal type 5, TARC; Thymus and activation-regulated chemokine, MDC; Macrophage-Derived Chemokine, CTACK; Cutaneous T cell-attracting chemokine, PARC; Pulmonary and activation-regulated chemokine, DPP-4; Dipeptidyl peptidase-4, ECP; Eosinophil cationic protein, LDH; Lactate dehydrogenase, CA2; Carbonic anhydrase II, NELL2; Nel-like protein 2, EDN; Eosinophil-derived neurotoxin, SCCA2; Squamous Cell Carcinoma Antigen 2, FABP5; Fatty acid binding protein 5, VEGF; Vascular endothelial growth factor).

clinical data, RAB25 was co-expressed with filaggrin, indicating that RAB25 is a promising severity biomarker that reflects the key pathogenesis of AD.[54]

Collecting skin, blood, or cord tissue samples from AD patients is theoretically straightforward for biomarker research, but large-scale cohort studies are limited due to the challenges of conducting prospective research and concerns over invasive methods. To overcome the invasiveness of the classical route of sample acquisition, non-invasive sampling methods, including tape stripping and microneedle patches, have been actively investigated to capture not only the immunologic landscape of AD, but also to predict the development of AD. Recently, Andersson et al. showed the possibility of tape stripping to capture the immunologic biomarkers comprising TARC and CTACK, which are suggested to correlate better with the clinical severity of AD.[22] In addition, another study showed that tape stripping from 2-month-old infants could be used to predict the development of AD.[63] Besides tape stripping which gathers information mainly from the stratum corneum, a hyaluronic acid-loaded microneedle patch (HA-MN) is suggested as a non-invasive skin sampling technique. Lee et al. showed that the HA-MNs are better to tape stripping in aspect of the acquisition of proteins from skin.[64] Also, the hallmark Th2 cytokines (e.g., IL-4, IL-13) were easily detected with HA-MNs because the penetration of microneedles reach up to 650 μm without pain.

Besides of the biomarkers with immunologic signatures, sensitisation to commensal yeasts in the skin, such as *Malassezia*, also can be used to detect disease severity in AD, indicating that the *Malassezia*-specific IgE may be a potential severity biomarker.[48] Also, sensitization to self-proteins has been thought to cause AD, which is distinct from the classic AD clinical phenotype.[65] Nonetheless, the utility of these biomarkers is viewed differently in clinical trials and clinical settings, since the clinical efficacy of particular therapies for AD is best measured by clinicians and patients through visual assessments.

Monitoring biomarkers

Despite the heterogeneity in clinical phenotypes of AD, chronicity and recurrence are common to all subtypes. As a result, multiple monitoring biomarkers have been proposed to track disease activity over time, reflecting AD's natural progression. Serum total IgE is the most frequently evaluated serological marker in clinical settings and clinical trials,[28] yet its levels can be normal in patients with intrinsic AD, indicating it may not be the best indicator of disease activity or severity. Besides serum total IgE level, eosinophilic cationic protein (ECP) and TARC have both been identified as biomarkers for assessing disease activity.[50] Specifically, TARC is currently the most accurate and efficient biomarker. The pooled correlation coefficient of longitudinal randomized trials was 0.6 [95% confidence interval (CI), 0.48–0.70], while the pooled correlation coefficient of cross-sectional studies was 0.64 [95% CI, 0.57–0.70], according to a recent meta-analysis.[28] This analysis also highlighted serum CTACK, E-selectin, MDC, LDH, and IL-18 as promising monitoring biomarkers for clinical use.[28]

Although TARC levels are known to correlate with AD disease activity, serum TARC levels vary among patients with the same severity score. Individual variations in TARC levels are believed to result from the diverse pathogenic processes involved in AD. To overcome the limitation of single biomarker, a recent pilot study involving 17 AD patients developed a biomarker panel including TARC, PARC, IL-22, and soluble IL-2R, showing a high correlation (0.86) with disease severity.[29] This panel was further validated in larger cohorts to assess the effectiveness of various AD therapies.[30] Likewise, several other studies have shown that biomarker combinations, reflecting multiple immunological pathways, correlate more closely with AD severity than individual markers.[38] Hence, employing a panel of biomarkers offers a more accurate approach to monitoring AD, capturing its complex pathogenesis. Although serological samples are convenient for collecting, analysing, and interpreting biomarkers, skin lesions contain most of the crucial data regarding the pathogenesis of AD. Recent studies have focused on changes in the skin transcriptome following narrowband ultraviolet B, cyclosporine administration, and dupilumab treatment.[52,66,67]

Severe chronic pruritus is an important factor in determining the severity of AD. Due to the itching-scratching vicious cycle, pruritus can cause sleep disruptions and poor daily life function, lowering the quality of life of patients with AD and worsening the severity of skin lesions. While various questionnaires are used in clinical trials and real-world settings to assess itching and the resultant deterioration in patient quality of life, there is no tool for objectively measuring itching with high reproducibility. Thus, IL-31, which is known to be a major driver of pruritus in AD,[42,43,68] is intended to be used as a pruritus biomarker. The association between disease activity and serum IL-31 levels has been studied in several studies,[69] but its correlation with itching is yet to be investigated; therefore, further research is required to ascertain the correlation between pruritus and serum IL-31 levels.

Preventive, prognostic biomarkers

According to a recent Delphi survey of international experts, preventive and prognostic biomarkers hold the highest importance in managing AD.[70] Epidemiological studies suggest that the disease's progression and related comorbidities, like AM, may only affect certain patient subgroups.[71] There is critical need for biomarkers that can offer essential insights into the

disease's trajectory from childhood, such as predicting remission before adolescence or ongoing chronic inflammation, and notably, the development of allergic asthma. Furthermore, as AD can be a lifelong condition, biomarkers that foresee disease stages would be invaluable for preventing AD in older adults and addressing potential comorbidities.

Davidson et al. highlighted the current shortage of dependable biomarkers for identifying individuals at risk of progressing through AM.[72] Despite differing methodologies, various cohort studies consistently identify risk factors for AM, including filaggrin mutations, polysensitisation, extended AD duration, familial atopic history, early onset, and severe disease phenotype.[71] This consistency across studies indicates that further research into the AD-AM link could greatly enhance our knowledge of type 2 immune responses in various organs.

Possible candidate biomarkers for the prediction of AM are currently under investigation (Table 2). Recently, fatty acid-binding protein 5 (FABP5) was suggested as a biomarker to predict the progression of AD to AM.[73] The study found that in AD patients progressing to AM, there's an increase in gene expression related to fatty acid metabolism, particularly FABP5 genes, compared to AD patients without AM and healthy controls. This elevated FABP5 expression was consistent across human skin samples and T cells from AM subjects, as well as in mouse models of AM, alongside higher IL-17A levels. Experimentally reducing FABP5 levels led to decreased IL-17A expression in T cells, indicating a direct relationship between FABP5 and IL-17A. This suggests that FABP5's role in enhancing Th17 responses could be a key factor in AM progression.[73]

A recent study suggests that immunoglobulin E (IgE) autoantibodies could be biomarkers for AM.[65] This cross-sectional study found that AD patients with other type 2 comorbidities had higher levels of autoreactive IgE compared to those with only AD. Notably, a significant majority of AD patients with elevated autoreactive IgE also had type 2 comorbidities. Moreover, these patients were younger and had higher total serum IgE levels, suggesting a potential connection between autoreactive IgE and AM's pathophysiology.[65] However, further research is needed to understand how autoreactive IgE develops and its role in AM.

Exploring oxidative stress as a basis for predicting AM involves assessing reactive oxygen species (ROS), which can trigger tissue inflammation, damage to epithelial barriers, and production of pro-inflammatory cytokines.[74] Several oxidative biomarkers have been identified, such as serum malondialdehyde (MDA), urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG), urinary acrolein-lysine, and bilirubin oxidative metabolites.[75] Additionally, the thiol-disulfide balance in AD patients tends towards oxidation, indicated by a higher disulfide/total thiol ratio and a lower native thiol/total thiol ratio.[76] In particular, exhaled breath condensate (EBC) of AD patients shows a reduced pH and increased leukotriene B4 and 8-isoprostane levels, markers of lipid peroxidation.[77] Other potential biomarkers for AM include serum levels of vascular endothelial growth factor (VEGF)[78] and elevated IL-13 levels.[79] However, these studies must be confirmed by large-scale studies.

Biomarkers	Evidence	References
FABP5, IL-17A	Whole genome transcriptome between AD, AD with AM, AD without AM. Increased level of FABP5 in AD with AM group was identified.	73
Oxidative stress markers	Increased malondialdehyde Increased urinary 8-hydroxy-2′-deoxyguanosine Increased urinary acrolein-lysine Increased bilirubin oxidative metabolites Increased disulfide/total thiol ratio Decreased native thiol/total thiol ratio Increased LTB4 in exhaled breath condensate Increased 8-isoprostane in exhaled breath condensate Decreased pH in exhaled breath condensate	74–77
VEGF	Increased serum VEGF level at three years	78
IL-13	Increased serum IL-13 level	79
GWAS with unsupervised modeling	Black race: chromosome 2–rs60242841; AD to AA White race–male/female: chromosome 13–rs9565267; AD to AR White race–male: chromosome 4–rs 151041509; AD to AR White race–female: chromosome 17–rs 78171803; AD to AR Asian race–female: no associated SNP; AD to FA	80
Immunoglobulin E (IgE) autoantibody	AD patients with AA or AR or FA showed higher prevalence of IgE autoantibodies. AD patients with IgE autoantibodies were younger, and displayed higher total serum total IgE level	65

FABP5, fatty acid binding protein 5; AD, atopic dermatitis; AM, atopic march; LTB4, leukotriene B4; VEGF, vascular endothelial growth factor; GWAS, genome-wide association; AA, allergic asthma; AR, allergic rhinitis; FA, food allergy.

Table 2: **Current candidate biomarkers for predicting AM progression.**

In addition to serum/skin biomarkers, a genome-wide association with an unsupervised modelling approach has been used to develop genotypic biomarkers of AM. Stanislaw et al. recruited 158,510 medical records of paediatric primary care patients. Demographic features were linked to allergic diseases using hierarchical clustering and decision tree modelling. As a result, race-specific AM comorbidities and risk loci could be identified.[80]

The biomarkers reflecting novel molecular endo-phenotypes of AD

Over the last decade, there's been significant progress in creating therapies that specifically target Th2 immune responses in AD. This has spurred interest in identifying treatments that, by blocking certain cytokines, offer better efficacy or fewer side effects for specific AD endotypes and phenotypes. This marks a move towards applying precision medicine concepts to AD, with ongoing research focused on finding reliable biomarkers to accurately identify AD endotypes (Fig. 2). Periostin is an extracellular matrix protein whose expression is boosted by Th2-induced IL-4 and IL-13, which are hallmark immune responses in AD. A recent study reported that serum IL-13, periostin, and dipeptidyl peptidase (DPP)-4 levels may be used to infer the systemic impact of skin inflammation on AD. In this study, a significant number of patients with low EASI scores (<16) exhibited elevated levels of these biomarkers. These findings could aid in identifying patients with particular subtypes of the disease, potentially correlated with their treatment responses to IL-13 targeted therapies.[39] Also, elevated baseline levels of IL-22 in skin tissues are being considered as a potential biomarker for predicting responses to IL-22 inhibitor treatment (fezakinumab).[37] Another study which used meta-analysis approach,[81] the CCL22 expression in the

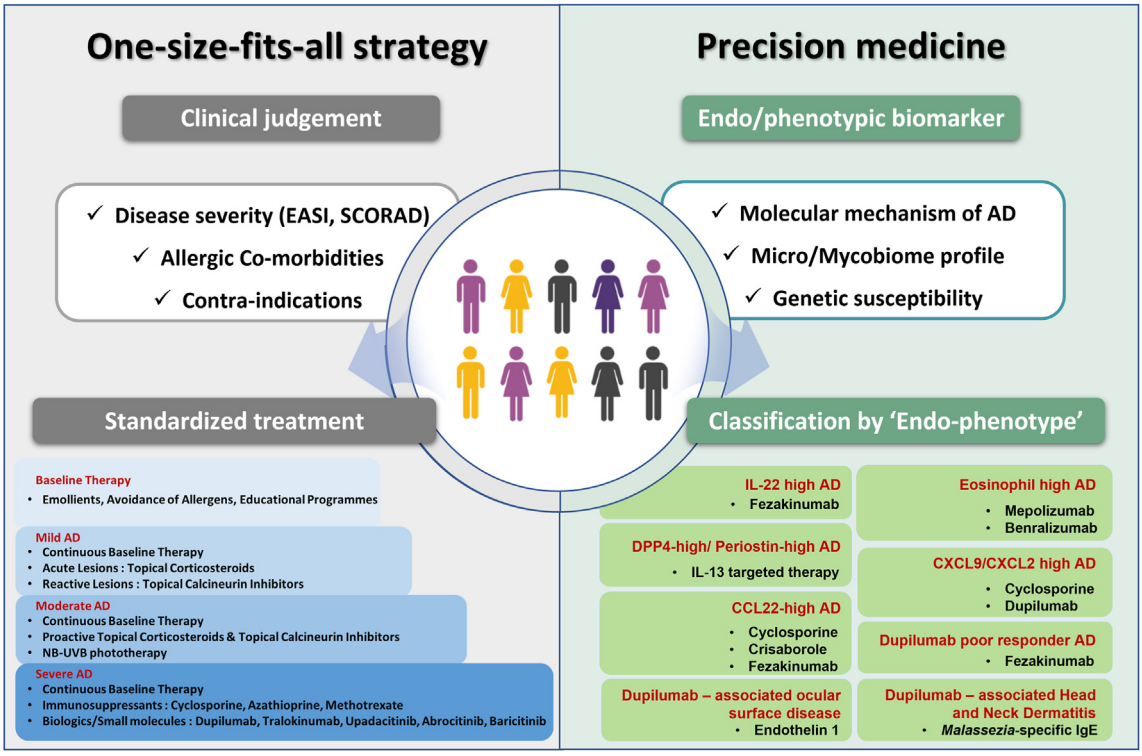

Fig. 2: Endo/phenotypic classification with biomarkers could enable the application of "precision medicine" concept in AD. The left panel represents the current 'one-size-fits-all' strategy for AD management. Clinical judgment according to disease severity with visual inspection of the skin lesions (EASI, SCORAD), consideration of comorbidities, and other complications which could limit the use of systemic immunosuppressants are primarily used in current clinical environments. After the assessment of those factors, management strategy usually follows the standardized protocol which mainly consider the disease severity only. This approach could neglect the individual characteristics of immunological pathophysiology which might differ between various disease endo/phenotypes. The right panel represents the application of "precision medicine" concept in AD. By considering multiple aspects of AD pathogenesis, including the molecular immunologic mechanisms of AD development, micro/mycobiome profiles, and genetic susceptibility, patients might be accurately differentiated. Although the validation is needed with large cohort study, several endo/phenotypic biomarkers which could differentiate the AD patients for the specific therapeutic options have been proposed. Those endo/phenotypic classifications and biomarkers could be used to enable the personalized treatment of AD. (EASI; The Eczema Area and Severity Index, SCORAD; SCORing Atopic Dermatitis).

Search strategy and selection criteria

Data for this Review were identified by searches of MEDLINE, PubMed and references from relevant articles using the search terms "endotypes of AD", "endophenotypes of AD", "phenotypes of AD", "precision medicine in AD". "Biomarkers of atopic dermatitis", "biomarkers of atopic march", "biomarkers of allergic diseases", "pathogenesis of AD", "pathogenesis of atopic march", "Immune response in AD", "Biologics in allergic diseases", "Biologics in AD". Abstracts and reports from meetings were included only when they were directly related to previously published studies. Only the articles published in English between 1990 and 2024 were included.

skin tissues were found to be the most reliable biomarker to predict the treatment response with cyclosporine, topical crisaborole, and fezakinumab. The same literature suggested that CXCL9 (associated with Th1/interferon responses) and CXCL2 (related to Th17 responses) as specific predictive biomarkers for treatments with cyclosporine and dupilumab, respectively.[81] A recent meta-analysis of clinical trials with biologics for AD indicated higher baseline IL-13 levels could predict better dupilumab response. Additionally, the study suggested that the poor dupilumab responders might benefit from additional suppression of the IL-22 pathway.[82] According to another recent study, CCL26/eotaxin-3 and SCCA2 were found to be the best representer of EASI, and LDH best reflected the POEM and pruritus-NRS.[33] These study group were also tried to delineate the biomarkers for dupilumab-associated ocular surface disease. They suggested that ET-1 (endothelin-1) were associated with the development of dupilumab-associated blepharitis and conjunctivitis.[83] Likewise, AD patients who experience dupilumab-associated head and neck dermatitis showed elevated *Malassezia*-specific IgE, which might be used as biomarker to determine the administration of dupilumab.[49] Consequently, with the advancement of molecularly targeted therapies, research is actively exploring various biomarkers that can classify the endotypes of AD, guiding the selection of specific targeted medications.

Not only studies that utilize follow-up of biomarker levels in cohorts, data-driven analysis have been tried to delineate the endotypes and phenotypes of AD. Thijs et al. reported the results of a principal component analysis (PCA) of adult patients with AD, identifying four distinct clusters of AD patients.[84] Another study employing a prediction model found that just ten biomarkers were necessary to classify paediatric AD patients into four distinct endotypes. Of these, PARC/CCL18 and apelin were most closely linked to persistent AD.[85] A study analysing adult AD blood samples with 131 markers through high-throughput proteomic analysis successfully categorized AD patients into two groups: a "high inflammatory group" and a "low inflammatory group." "High inflammatory group" showed elevated levels of pro-inflammatory cytokines, including TNF-β, MCP-3, and IL-13, and was associated with higher disease severity.[86] Most recent study by Möbus et al.[51] examined the blood transcriptomics from 60 adult AD patients. Using unsupervised learning, AD patients were classified into two clusters, differing mainly in eosinophil signalling transcripts (eosinophil-high endotype, eosinophil-low endotype). In eosinophil-high endotype, transcriptomic disturbances related to overall immune response were more pronounced, as well as a positive correlation between disease severity and IL-5 signitures were noted. In contrast, eosinophil-low endotype showed fewer transcript disturbances and no correlation between disease severity and IL-5 signitures. These outcomes suggest a specific endotype could be effectively managed with antibodies targeting eosinophil-related cytokines (IL-5). Recent trials with mepolizumab/benralizumab which target IL-5 didn't show significant efficacy in moderate-to-severe AD patients.[87,88] However, the study findings underscore the necessity of conducting large-scale clinical trials incorporating endotypes based on eosinophil (IL-5 signalling signature) status.

Conclusions and future perspectives

Although various endophenotypes of AD have been elucidated, diagnosis and treatment still rely on uniform criteria. This method adopts a 'one-size-fits-all' approach rather than predicting individual treatment responses. Moreover, classical approach can lead to unnecessary costs for both patients and healthcare systems, potentially cause avoidable side effects, and increase dissatisfaction with AD care.

In this context, identifying biomarkers as diagnostic standards and developing endophenotypes are crucial in the era of precision medicine. Various biomarkers have been developed, including the creation of entirely new biomarkers and biomarker panels in response to the development of 'omics' results. Biomarkers from the blood, skin tissue, and genetic variations, as well as biomarker panels that combine multiple forms of biomarkers, will be used to classify patients with AD into different endophenotypes in near future. This will pave the foundation for precision medicine based on advances in the understanding of the heterogeneous pathogenesis of AD.

Contributors

Chang Ook Park: writing-original draft, conceptualisation, investigation, project administration.

Su Min Kim: writing-original draft, investigation, methodology, visualisation.

Kwang Hoon Lee: conceptualisation, supervision, investigation, methodology, project administration, writing-review, and editing.

Thomas Bieber: conceptualisation, supervision, investigation, methodology, project administration, writing-review, and editing.

All authors read and approved final version of the manuscript and offered significant intellectual contribution.

Outstanding questions

1. Can biomarkers representing the endotypes and/or phenotypes of AD be used to select appropriate medications for specific patient subpopulations?
2. Can biomarker panel-based diagnostic algorithms of AD be developed in the near future?
3. Can biomarkers which aim to predict AM development be utilised in the clinical field?

The originality of figures and tables

The figures and tables in this manuscript are original, have not been published previously, and do not require permission for publication.

Declaration of interests

Dr. Bieber reports personal fees from AbbVie, Affibody, Almirall, Amagma, AnaptysBio, AOBiom, Anergis, Apogee, Arena, Aristea, Artax, Asana Biosciences, ASLAN pharma, Astria, Attovia, Bayer Health, Biofilm control, BioVerSys, Böhringer-Ingelheim, Bristol-Myers Squibb, BYOME Labs, Connect Pharma, Daichi-Sanyko, Dermavant, DICE Therapeutics, Domain Therapeutics, DS Pharma, EQRx, Galderma, Galapagos, Glenmark, GSK, Incyte, Innovaderm, Janssen, Kirin, Kymab, LEO, LG Chem, Lilly, L'Oréal, MSD, Medac, Micreos, Nektar, Novartis, Numab, OM-Pharma, Overtone, Pfizer, Pierre Fabre, Q32bio, RAPT, Samsung Bioepis, Sanofi/Regeneron, TIRmed, UCB, Union Therapeutics, UPStream Bio, YUHAN, other from founder and chairman of the board of the non-profit biotech "Davos Biosciences AG" within the international Kühne-Foundation, personal fees from founder of the consulting firm "Bieber Dermatology Consulting", outside the submitted work.

Dr. Park has nothing to disclose.

Dr. Lee has nothing to disclose.

Dr. Kim has nothing to disclose.

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