Shakir *et. al.,* Babcock Univ. Med. J.2025 8(2):310-318
https://doi.org/10.38029/babcockuniv.med.j..v8i2.1075
eISSN: 2756-4657

RESEARCH ARTICLE OPEN ACCESS

CD27 and IL-35 as diagnostic biomarkers in alopecia areata: First evidence of association with pathogenic scalp bacterial infections

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Submitted: 29th May 2025
Accepted: 2nd August 2025
Published: 31st December 2025
ID: Orcid ID

Abstract
Objective: This study aimed to investigate the diagnostic and prognostic potential of CD27 and interleukin-35 (IL-35) in patients with Alopecia Areata (AA), with and without pathogenic bacterial infections. To our knowledge, this is the first study to correlate CD27 and IL-35 expression with bacterial colonisation in AA.
Methods: A total of 60 patients with clinically diagnosed AA and 60 age- and sex-matched healthy controls were recruited. Blood samples were analysed for CD27 and IL-35 using ELISA. Scalp swabs from AA patients were cultured on blood agar, MacConkey agar, and Mannitol salt agar; bacterial isolates were identified through biochemical assays and confirmed by the VITEK 2 Compact System. Bacteria were deemed pathogenic if colony-forming unit concentrations exceeded 10^3–10^4 CFU/mL. Statistical analysis included ANOVA, Pearson correlation, and ROC curve analysis.
Results: Among AA patients, 43.3% showed bacterial growth; 12 isolates were classified as pathogenic. The most common species were Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, and Staphylococcus haemolyticus. CD27 and IL-35 levels were significantly elevated in AA patients with bacterial infections compared to those without bacterial infections and to healthy controls ($p = 0.001$). A strong positive correlation was observed between CD27 and IL-35 in infected patients ($r = 0.742$). CD27 showed perfect diagnostic performance (AUC=1.00), while IL-35 was a strong predictor in infected cases (AUC = 0.94).
Conclusion: CD27 and IL-35 may serve as useful immunological biomarkers in AA. Elevated IL-35 levels appear to be driven by bacterial-induced immune activation, suggesting a potential role for combined immunomodulatory and antimicrobial therapies.

Keywords: Alopecia Areata, CD27, Interleukin-35 (IL-35), Scalp Microbiota, Biomarkers

Plain English Summary

Alopecia Areata (AA) is a condition where the body's immune system mistakenly attacks the hair follicles, leading to sudden and often distressing hair loss. While the exact cause is unknown, both the immune system and scalp bacteria may play a role in how the disease develops or worsens. In this study, we looked at two molecules in the blood, CD27 and IL-35, that are involved in how the immune system responds to threats. We wanted to see if these molecules could help identify people with AA and whether their levels change when harmful bacteria are present on the scalp. We studied 60 people with AA and 60 healthy individuals. We tested their blood for CD27 and IL-35, and we also took swabs from the scalp to check for bacteria. We found that nearly half of the AA patients had bacteria growing on their scalps, most commonly Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa. We discovered that CD27 levels were much higher in all AA patients, and IL-35 levels were

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especially high in those with bacterial infections. This suggests that the immune system becomes more active when harmful bacteria are present. These two markers could help doctors better understand a patient's condition and possibly decide whether both immune and antibiotic treatments are needed. This is the first study to explore how these immune markers relate to bacterial infections in AA, offering new insight into how the disease might be managed more effectively.

Introduction

Alopecia Areata (AA) is a prevalent, chronic, tissue-specific autoimmune disorder characterised by non-scarring hair loss, typically manifesting as round or oval bald patches on the scalp or other hair-bearing areas of the body (1). Affecting nearly 2% of the global population, AA commonly presents in young adults and significantly impacts psychological well-being due to its sudden and often visible nature (2). Based on the extent of hair loss, AA can be clinically categorised into three types: Patchy AA, Alopecia Totalis (complete scalp hair loss), and Alopecia Universalis (loss of all body hair) (3).

The pathogenesis of AA is primarily attributed to the collapse of the hair follicle's immune privilege, a protective mechanism that normally shields it from autoimmune attack (4). This breakdown leads to the exposure of follicular autoantigens, triggering infiltration by autoreactive T cells and the release of various proinflammatory cytokines. Among these immune modulators, CD27 and IL-35 have emerged as potentially important biomarkers in autoimmune and inflammatory processes.

CD27 is a type I transmembrane glycoprotein that belongs to the tumour necrosis factor receptor (TNFR) superfamily (5). It is constitutively expressed on natural killer (NK) cells, helper T cells, cytotoxic T cells, and hematopoietic stem cells (6). The soluble form of CD27 (sCD27) may enhance the activation of T cells and antigen-primed B cells, leading to increased production of immunoglobulin G (IgG) (7). This signalling pathway has been associated with heightened immune responses in several autoimmune diseases.

Interleukin-35 (IL-35), on the other hand, is a potent anti-inflammatory cytokine and a relatively recent addition to the IL-12 family (8). It is secreted by regulatory T cells (Tregs), regulatory B cells, and other cell types such as macrophages, dendritic cells, and endothelial cells. IL-35 is known to suppress Th1 and Th17-mediated immune responses, and its deficiency may contribute to the progression of autoimmune diseases (9).

In addition to immune dysfunction, recent evidence has suggested that microbial dysbiosis may play a role in AA pathogenesis. The scalp microbiota is critical in maintaining skin immune homeostasis, and its disruption by pathogenic bacteria can potentiate local inflammation and immune activation targeting the hair follicles (10, 11). Bacterial infections are known to modulate cytokine expression, further influencing the immune response (12). However, the interaction between microbial pathogens and immunological markers such as CD27 and IL-35 in AA remains underexplored.

In this context, the present study aims to investigate the levels of CD27 and IL-35 in patients with AA and examine their correlation with the presence or absence of pathogenic bacterial infections. This study was conducted in the Najaf Governorate and is the first to explore these biomarkers as potential diagnostic and prognostic indicators in AA patients with bacterial co-infections.

Materials and Methods

Study Design and Participants

This case-control study was conducted from October 2024 to March 2025. A total of 120 individuals were recruited, comprising 60 patients diagnosed with Alopecia Areata (AA) and 60 healthy control subjects. Participants were enrolled from the Dermatology Consultation Unit at Al-Najaf Teaching Hospital and Al-Sader Medical City in Al-Najaf Al-Ashraf Governorate, Iraq.

Sample Size Justification

The sample size of 60 AA patients was determined based on preliminary data and previous studies reporting medium-to-large effect sizes for immune biomarker differences in autoimmune dermatological conditions. A power analysis ($\alpha = 0.05$, power = 0.80) for detecting a significant difference in cytokine levels between groups indicated that a minimum of 52 subjects would be adequate. To accommodate possible dropouts and improve generalizability, a total of 60 patients were included.

Inclusion criteria for AA patients:

Clinical diagnosis of Alopecia Areata (patchy, totalis, or universalis); Aged 11–70 years; and no immunosuppressive treatment in the last 6 weeks.

Exclusion criteria

Presence of other autoimmune, infectious, or chronic inflammatory diseases and recent antibiotic therapy (<30 days).

Control group selection
Healthy individuals (n=60) were matched for age and sex with AA patients. Control participants were screened to exclude any visible scalp lesions, recent hair loss, or history of dermatological or autoimmune conditions. Scalp swabs were taken from all controls to confirm the absence of microbial dysbiosis.

Data Collection
All participants completed a structured questionnaire capturing demographic data (age, sex) and clinical variables (AA subtype, severity, family history, nail involvement).

Blood Collection and Cytokine Measurement
Five millilitres of venous blood were collected from each participant using sterile techniques. Samples were centrifuged at 2,500 rpm for 10 minutes to separate serum. Serum levels of CD27 and IL-35 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (BT LAB, China), following the manufacturer's protocol. All samples were assayed in duplicate to ensure consistency.

Scalp Swab Collection and Microbiological Analysis
Sterile swabs were used to gently sample the affected scalp areas (or corresponding sites in controls). Each swab was cultured on the following media: Blood Agar, MacConkey Agar, and Mannitol Salt Agar (MSA). Plates were incubated at 37°C for 24–48 hours. Colony morphology, haemolysis patterns, and fermentation capabilities were assessed. Bacterial identification was confirmed through standard biochemical testing (MacFaddin, 2000) (13) and further verified using the VITEK 2 Compact System, with identification probabilities ranging from 85% to 99%.

Definition of Pathogenic Bacteria
Bacterial isolates were classified as pathogenic if the colony-forming unit (CFU) concentration exceeded 10^3–10^4 CFU/mL, based on diagnostic criteria outlined by Carroll et al. (14). This threshold distinguishes between normal skin microbiota and clinically relevant pathogens. Non-pathogenic (commensal) isolates were excluded from subsequent analyses.

Statistical Analysis
Statistical analyses were conducted using IBM SPSS version 28 (15). The normality of data was assessed using the Shapiro-Wilk test. Descriptive statistics were reported as means ± standard deviations for continuous variables and as frequencies and percentages for categorical variables. Group comparisons were performed using one-way ANOVA for continuous variables and chi-square tests for categorical data.
Pearson's correlation coefficient was used to evaluate relationships between CD27 and IL-35 levels. Receiver Operating Characteristic (ROC) curve analysis was employed to determine the sensitivity and specificity of each biomarker. A p-value < 0.05 was considered statistically significant.

Results

Clinical and Demographic Characteristics of AA Patients
A total of 60 patients diagnosed with Alopecia Areata (AA) were included in the study. Table 1 summarises the key clinical characteristics. Male participants (n=53, 88.33%) significantly outnumbered females (n=7, 11.66%) (p=0.001). The most affected age group was 21–30 years (43.33%), followed by 31–40 years (23.33%).
In terms of disease severity, 65% of patients presented with mild-to-moderate AA, while 35% had severe involvement (p=0.017). Patchy AA was the predominant subtype (85%), with much lower occurrences of Alopecia Universalis (13.33%) and Totalis (1.66%) (p=0.001). Most patients (76.66%) showed no nail changes, a statistically significant difference (p<0.0001).

Table 1: Clinical characteristics in patients with Alopecia Areata (n=60)

Characteristic	Category	n	%	p-value
Sex	Male	53	88.33%	0.001
	Female	7	11.66%	
Age group (years)	11–20	10	16.66%	–
	21–30	26	43.33%	
	31–40	14	23.33%	
	41–50	5	8.33%	
	51–60	3	5.00%	
	61–70	2	3.33%	
Severity	Mild to Moderate	39	65.00%	0.017
	Severe	21	35.00%	

Type of AA	Patchy	51	85.00%	0.001
	Totalis	1	1.66%	
	Universalis	8	13.33%	
Nail involvement	Without nail changes	46	76.66%	<0.0001
	With nail changes	14	23.33%	

Bacterial Culture Results

Out of 60 scalp swabs collected from AA patients, 26 samples (43.3%) exhibited bacterial growth, whereas 34 samples (56.6%) showed no growth (Figure 1).

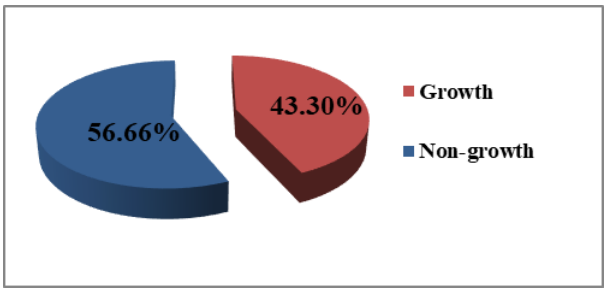

Figure 1: Bacterial growth

Among the culture-positive samples, 12 isolates (46.15%) were identified as pathogenic bacteria, defined by exceeding a concentration threshold of 10^3–10^4 CFU/mL (14). The remaining isolates were classified as commensal microbiota and excluded from analysis (Table 2).

Table 2: Pathogenic bacterial isolates from AA patient samples

Bacterial Species	No. of Isolates	Percentage (%)
Staphylococcus aureus	3	25
Streptococcus pyogenes	2	16.66
Staphylococcus haemolyticus	2	16.66
Pseudomonas aeruginosa	2	16.66
Bacillus cereus	1	8.33
Staphylococcus xylosus	1	8.33
Streptococcus downei	1	8.33
Total	12	100

Pathogen identification was confirmed using VITEK 2 Compact System

Cultural and Biochemical Characteristics of Pathogenic Bacteria

Cultural morphology, haemolysis, growth on selective media, and biochemical test results for the 12 pathogenic isolates are summarised in Table 3.

Table 3. Combined cultural and biochemical characteristics of pathogenic bacterial isolates

Bacterial Isolate	Gram Stain	Haemolysis	Mannitol Fermentation	Catalase	Oxidase	Coagulase	Motility
S. aureus	+	β	+	+	–	+	–
S. haemolyticus	+	β	–	+	–	–	–
S. xylosus	+	γ	–	+	–	–	–
S. pyogenes	+	β	–	–	–	–	–
S. downei	+	α	–	–	–	–	–
B. cereus	+	β	–	+	+	–	+
P. aeruginosa	–	β	–	+	+	–	+

All isolates showed consistent colony morphology as described in the original Table 3

Serum Biomarker Levels (CD27 and IL-35)

Serum concentrations of CD27 and IL-35 were compared across three groups: AA patients with bacterial infections, AA patients without bacterial infections, and healthy controls. Results are presented in Table 4. CD27 levels were

significantly higher in AA patients with bacterial infections (881.51 ± 0.072 ng/mL) compared to those without infections (322.65 ± 0.086 ng/mL) and controls (13.94 ± 0.017 ng/mL) (p=0.001). IL-35 levels were also elevated in AA patients with infections (14.94 ± 0.001 ng/mL), whereas patients without infections had lower levels (8.28 ± 0.007 ng/mL). Controls showed intermediate levels (11.09 ± 0.02 ng/mL) (p=0.001).

Table 4: CD27 and IL-35 serum levels in AA patients and controls

Group	CD27 (ng/mL)	IL-35 (ng/mL)
AA + Pathogenic Bacteria	881.51 ± 0.072	14.94 ± 0.001
AA – Bacteria	322.65 ± 0.086	8.28 ± 0.007
Healthy Controls	13.94 ± 0.017	11.09 ± 0.020
p-value	p=0.001	p=0.001

Correlation Analysis

A strong positive correlation was observed between CD27 and IL-35 in AA patients with bacterial infections (r=0.742, p=0.001). Conversely, a moderate negative correlation was found in AA patients without bacterial infections (r=–0.536, p=0.001), as shown in Table 5.

Table 5. Correlation between CD27 and IL-35 in AA patients

Group	Correlation (r)	p-value
With Bacterial Infections	0.742	p=0.001
Without Infections	–0.536	p=0.001

Receiver Operating Characteristic (ROC) Analysis

ROC curve analysis evaluated the diagnostic accuracy of CD27 and IL-35 as biomarkers for AA, particularly in distinguishing bacterial infection status. Table 6 and Figure 2 present the results with exact p-values and 95% confidence intervals (CI) for the area under the curve (AUC). CD27 showed excellent diagnostic performance with an AUC of 1.00 (CI: 1.00–1.00), 100% sensitivity, and 100% specificity. IL-35 was also a strong predictor in AA patients with infections (AUC=0.94, CI: 0.885–1.000), while its diagnostic value diminished without infection (AUC=0.56, CI: 0.255–0.656).

Table 6: ROC curve analysis of CD27 and IL-35 in AA patients with bacterial infections

Biomarker	Cut-off	AUC	95% CI	Sensitivity	Specificity	p-value
CD27	>2.108	1	1.000–1.000	100%	100%	0
IL-35 (Infected AA)	>3.00	0.94	0.885–1.000	88%	97%	<0.001
IL-35 (Non-Infected AA)	>1.99	0.56	0.255–0.656	77%	44%	0.347

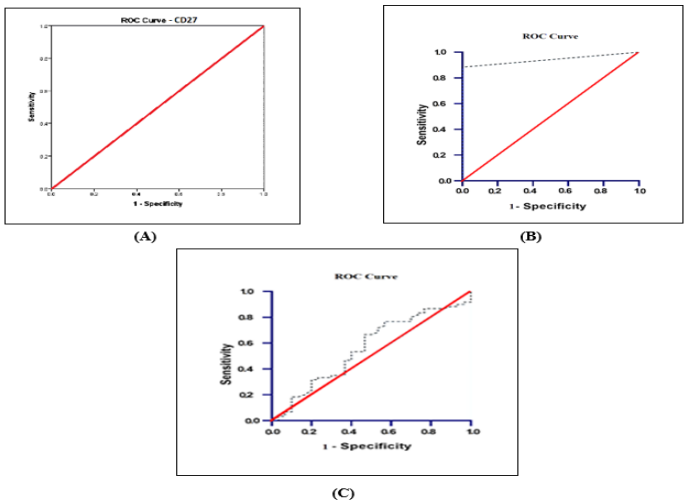

Figure 2: ROC curves for (A) CD27, (B) IL-35 in infected AA, and (C) IL-35 in non-infected AA
All figures are provided in high-resolution with axis labels and AUC values

Discussion

This study aimed to investigate the immunological markers CD27 and IL-35 in patients with Alopecia Areata (AA), with a particular focus on their association with pathogenic bacterial infections. The findings demonstrate that serum levels of both CD27 and IL-35 were significantly elevated in AA patients with bacterial infections compared to both AA patients without infections and healthy controls. These results highlight a potential diagnostic and prognostic role for these biomarkers in identifying immune activation linked to microbial dysbiosis in AA.

The demographic data aligned with previous epidemiological findings, showing a higher incidence of AA among males and individuals in their second and third decades of life (16, 17, 18). Psychological stress, hormonal influences (e.g., androgen–oestrogen receptor imbalance), and immune dysregulation are likely contributors to this age and sex distribution (16, 17). Mild-to-moderate disease severity and patchy AA were the most prevalent patterns, consistent with early-stage or less aggressive disease (19). The relatively low prevalence of nail involvement further supports that most cases in this cohort were in earlier stages of immune-mediated damage (20).

CD27 and IL-35 in Immune Activation and Autoimmunity

CD27, a costimulatory receptor expressed on T and B cells, plays a critical role in enhancing lymphocyte proliferation and survival during immune responses (6). Its soluble form (sCD27), detected in serum, reflects ongoing immune activation and has been associated with several autoimmune and inflammatory diseases (7). In the current study, CD27 levels were significantly higher in all AA patients, especially in those with coexisting bacterial infections. This supports the hypothesis that CD27 may not only reflect autoimmune activity but also amplify T-cell responses in the context of secondary microbial stimuli.

IL-35, a member of the IL-12 cytokine family, is typically produced by regulatory T cells (Tregs) and regulatory B cells and serves as a potent immunosuppressive cytokine (8, 9). Under physiological conditions, IL-35 helps maintain immune tolerance by suppressing effector T cell subsets (Th1 and Th17). However, its role in autoimmune contexts remains complex and at times paradoxical. In our study, IL-35 was significantly upregulated in AA patients with bacterial infections, but reduced in those without infections, suggesting that microbial exposure may modulate its expression.

This observation aligns partially with findings by Dixon et al. (9), who reported that IL-35 production by tolerogenic dendritic cells contributes to immune suppression in autoimmune environments. However, in the presence of microbial antigens, especially those containing pathogen-associated molecular patterns (PAMPs), IL-35 expression can become dysregulated. Toll-like receptor (TLR) engagement by bacterial components, such as lipopolysaccharide (LPS) in Gram-negative bacteria or lipoteichoic acid in Gram-positive organisms, can activate antigen-presenting cells and promote downstream cytokine cascades (21, 22). This may induce a compensatory increase in IL-35 as a mechanism to dampen inflammation. Thus, the elevated IL-35 levels observed in infected AA patients may reflect an attempt by the immune system to re-establish homeostasis following TLR-mediated immune activation.

Similarly, CD27 expression may be upregulated in response to microbial-derived danger signals. TLR activation enhances CD70-CD27 interactions, promoting T cell survival and memory differentiation. This could explain the synergistic elevation of both biomarkers in AA patients with secondary bacterial infections.

Microbial Dysbiosis and AA Pathogenesis

The link between microbial imbalance and AA has garnered growing attention. The presence of pathogenic bacteria on the scalp may act as a trigger for immune dysregulation through persistent antigenic stimulation and disruption of skin immune homeostasis (10, 11). Studies have demonstrated that pathogenic bacteria can induce inflammatory responses through the release of superantigens or by promoting Th1/Th17 differentiation, both of which are implicated in AA pathogenesis (22). In our study, common isolates such as Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa were identified, all of which are known to possess strong immunostimulatory properties.

The significant correlation between CD27 and IL-35 in infected AA patients suggests that microbial exposure may potentiate immune signalling pathways already active in autoimmune contexts. The ROC analysis further supports the diagnostic utility of these biomarkers, especially CD27, which demonstrated perfect sensitivity and specificity. While IL-35 showed strong performance in infected patients, its lower diagnostic power in non-infected AA highlights its context-specific regulation (23).

Implications and Future Directions

The findings of this study reinforce the potential utility of CD27 and IL-35 as diagnostic markers in

AA, particularly when evaluating patients with suspected bacterial superinfection. They also support a growing body of evidence linking scalp microbial imbalance with immune dysregulation in autoimmune hair loss. Future studies should adopt longitudinal designs, incorporate broader immunological profiling, and explore therapeutic interventions that target both immune pathways and microbial ecology.

From a therapeutic standpoint, CD27 and IL-35 could be integrated into a biomarker-guided treatment algorithm for AA. Elevated CD27 levels may indicate a need for immunomodulatory agents such as corticosteroids or JAK inhibitors, while concurrent elevation of IL-35, particularly in patients with confirmed scalp dysbiosis, could justify the addition of targeted antimicrobial therapy. This combined approach may offer enhanced control over inflammation and prevent disease exacerbation driven by bacterial triggers.

Study limitations

Despite these promising findings, several limitations must be acknowledged:

1. Cross-sectional Design: This study's design limits our ability to infer causality. While associations between bacterial presence and biomarker elevation were observed, longitudinal data are needed to determine whether microbial dysbiosis drives disease progression or arises because of immune dysfunction.
2. Confounding Variables: Although patients with recent infections or known autoimmune comorbidities were excluded, other potential confounders, such as diet, hygiene practices, or undisclosed medication use, may have influenced both microbial composition and immune markers.
3. Sample Size and Generalizability: While a power calculation was conducted, the study population was limited to a single geographic region (Najaf Governorate), which may limit the generalizability of results to other populations or ethnic groups.
4. Microbial Characterisation: The study focused on culture-based identification of bacteria. Molecular techniques (e.g., 16S rRNA sequencing) would allow more precise detection of microbial diversity and may identify unculturable pathogens relevant to immune modulation.
5. Single-Timepoint Cytokine Measurement: Serum levels of CD27 and IL-35 were measured only once per participant. Dynamic changes over time, particularly in response to treatment or fluctuating disease activity, remain unexamined.

Conclusion

This study provides evidence that the immunological markers CD27 and IL-35 are significantly elevated in patients with Alopecia Areata (AA), particularly in those with concurrent pathogenic bacterial infections. CD27 levels were consistently increased in all AA patients, reflecting underlying immune activation, while IL-35 was notably higher in the presence of bacterial colonisation, suggesting an adaptive immunoregulatory response to infection-induced inflammation. These findings support the potential of CD27 as a general diagnostic marker for AA and propose IL-35 as a complementary marker that may help distinguish infection-associated disease states.

The data also reinforce the emerging concept that microbial dysbiosis, particularly the overgrowth of pathogenic scalp bacteria, may exacerbate immune dysregulation in AA through mechanisms involving T-cell activation, Toll-like receptor signalling, and cytokine modulation. These pathways may lead to the upregulation of both proinflammatory and regulatory cytokines, including IL-35, as the immune system attempts to restore homeostasis. Understanding these interactions offers valuable insight into the immunopathogenesis of AA and opens avenues for biomarker-guided clinical assessment.

Based on these findings, we recommend that clinicians consider evaluating CD27 and IL-35 levels in AA patients, particularly in those with treatment-resistant or recurrent disease, where secondary infections may play a role. The inclusion of these biomarkers could improve diagnostic precision and inform decisions about the need for antimicrobial therapy or targeted immunomodulation.

Additionally, this study highlights the importance of maintaining scalp microbial homeostasis in managing AA. Regular scalp hygiene and microbiome-preserving therapies may offer non-pharmacological strategies to mitigate immune activation. Future studies should investigate whether interventions targeting pathogenic microbiota could complement existing immunotherapies in AA treatment.

Nevertheless, we caution that these conclusions are drawn from a cross-sectional study and further research, ideally longitudinal and multicentre in design, is needed to validate these markers in broader populations, establish causality, and assess their value in clinical decision-making.

List of Abbreviations

AA: Alopecia Areata
CD27: Cluster of Differentiation 27
IL-35: Interleukin 35
ELISA: Enzyme-Linked Immunosorbent Assay
TLR: Toll-like Receptor
LPS: Lipopolysaccharide
Th1: T helper cell type 1

Th17: T helper cell type 17
Treg: Regulatory T cell
PAMPs: Pathogen-Associated Molecular Patterns
ROC: Receiver Operating Characteristic
AUC: Area Under the Curve
CFU: Colony-Forming Unit
NK cells: Natural Killer cells
DCs: Dendritic Cells
MSA: Mannitol Salt Agar
ANOVA: Analysis of Variance
SPSS: Statistical Package for the Social Sciences
CI: Confidence Interval
IgG: Immunoglobulin G
S. aureus: Staphylococcus aureus
P. aeruginosa: Pseudomonas aeruginosa
B. cereus: Bacillus cereus
S. pyogenes: Streptococcus pyogenes
S. haemolyticus: Staphylococcus haemolyticus
S. xylosus: Staphylococcus xylosus
S. downei: Streptococcus downei
BT LAB: Bioassay Technology Laboratory (ELISA kit supplier)

Declarations

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Najaf Health Department. Written informed consent was obtained from all participants before enrolment. All procedures performed were following the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Consent for Publication

All authors have reviewed and approved the final version of the manuscript and consent to its publication under the terms of the relevant journal's license.

Availability of Data and Materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research received no external funding.

Authors' Contributions

SAN conceptualised the study, designed the methodology, performed data collection, and drafted the initial manuscript. ASM conducted the statistical analysis, interpreted the findings, and critically revised the manuscript. SSMH and ALS contributed to sample processing and laboratory analyses. All authors contributed to the study design, reviewed the final draft, and approved the submitted version.

Acknowledgments

The authors would like to thank the staff of Al-Najaf Teaching Hospital and Al-Sader Medical City for their support in patient recruitment and sample collection. Appreciation is also extended to the laboratory personnel who assisted in microbiological and immunological analyses.

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