Biobacta Journal of Biochemistry and Molecular Biology
https://bjbmb.org
BJBMB
SPBH

Original article

Evaluation of Th17 Cells, CD4+ T Cells, IL-17 and IL-22 in *Streptococcus Pyogenes*-Associated Psoriasis: An Integrated Immunological Study

Heyam Mohammed Al-Kenani

Department of Medical Microbiology, College of Medicine, University of Qadisiya, Iraq

Email: heam.muhmmad@qu.edu.iq

DOI: https://doi.org/10.71428/BJBMB.2026.0208

Abstract

Background: Psoriasis affects 2 to 3% of adults worldwide. Once primarily associated with older age and the male sex, emerging data indicate a U-shaped psoriasis prevalence curve. Th17 cells produce IL-17 and IL-22, which drive keratinocyte activation and inflammation, respectively. **Objective:** The current research aims to examine the correlation between S. pyogenes infection and Th17-mediated immune responses in psoriasis patients by assessing circulating Th17 and CD4+ T-cell populations and serum levels of IL-17 and IL-22. **Methods:** A case-control study is proposed involving patients with clinically diagnosed psoriasis and apparently healthy controls. Clinical severity was assessed using the Psoriasis Area and Severity Index (PASI). Evidence of *S. pyogenes* infection was determined using throat swab culture and/or species-specific PCR, with antistreptolysin O (ASO) antibody measurement considered as an additional marker of recent streptococcal exposure. Peripheral blood was analyzed by flow cytometry for CD4+ and Th17 cells, while serum IL-17 and IL-22 were quantified by ELISA. Associations between streptococcal status, immune biomarkers, and psoriasis severity were evaluated using correlation, regression, and ROC analyses. **Results:** Previous Iraqi data demonstrated significantly higher serum IL-17 concentrations in psoriasis patients than controls and significantly lower CD4+ levels. An independent Iraqi study involving 75 psoriasis patients and 75 controls also reported significantly increased serum IL-17 and IL-22, with IL-22 values of 42.23 ± 0.95 pg/mL in patients versus 18.07 ± 0.32 pg/mL in controls. **Conclusion:** Integration of environmental factors and microbial co-factors must be incorporated into cellular and cytokine immune response profiles in order to better understand psoriasis.

Keywords: psoriasis; Th17; CD4+ T cells; IL-17; IL-22; Streptococcus pyogenes; immunopathogenesis.

Introduction

Psoriasis is an inflammatory, chronic, and relapsing condition aggravated by rapid keratinocyte proliferation and intense activation of destructive cycles of both innate and adaptive immunity. Despite the complexity resulting from the interplay between genetics and the environment, current data point to the IL-23/Th17 axis as the principal danger pathway in psoriasis immunopathogenesis [1,2]. Synthesis of IL-23 by dendritic cells induces the activation and maintenance of Th17 cells. Th17 cells synthesize numerous cytokines, especially IL-17A, IL-17F, and IL-22, which act on keratinocytes and other skin cells to promote inflammatory responses and sustained psoriatic lesions [2,3]. IL-17A acts on keratinocytes, induces the synthesis of chemokines, activating cytokines, and antimicrobial peptides, thereby promoting the influx of inflammatory cells. The circulation of Th17 cells, dendritic cells, keratinocytes, and other immune cell types creates

Received: June 5, 2026. Accepted: August 12, 2026. Published: September 12, 2026

an amplifying inflammatory circuit that sustains psoriatic lesions [2,4]. The significance of the Th17/IL-17 pathway is corroborated by both experimental and clinical data. Elevated levels of IL-17-producing CD4+ T cells have been observed in the peripheral blood and affected skin of individuals with psoriasis. Besen et al. illustrated elevated levels of IL-17+ and IL-22+ CD4+ T cells in individuals with psoriasis and psoriatic arthritis, alongside heightened IL-17 production, indicating the participation of both T-cell subsets in psoriatic inflammation [5]. The clinical significance of the IL-23/Th17/IL-17 pathway is reinforced by the efficacy of treatments aimed at IL-17 and IL-23, which can lead to considerable enhancement in psoriasis severity [2,6].

IL-22 is a significant cytokine linked to Th17- and Th22-driven immune responses in psoriasis. In contrast to cytokines that mainly influence hematological cells, IL-22 primarily targets non-hematopoietic cells, such as keratinocytes. IL-22 stimulates keratinocyte growth, epidermal thickening, modified terminal differentiation, and the synthesis of antimicrobial proteins, therefore playing a role in various distinctive clinical traits of psoriasis [7]. Wawrzycki et al. documented markedly elevated circulating IL-22 levels in individuals with plaque psoriasis and established a positive association between IL-22 concentration and the Psoriasis Area and Severity Index (PASI), indicating a potential link between IL-22 and disease activity [7]. Consequently, the concurrent assessment of Th17 cells, IL-17, and IL-22 could yield a more thorough insight into the cellular and cytokine irregularities associated with psoriasis. Local data further corroborate the participation of these immune factors in psoriasis. In a prior case-control investigation conducted in Iraq, serum IL-17 levels were markedly elevated in 60 psoriasis patients compared to 60 healthy individuals (19.68 ± 5.31 vs. 13.23 ± 3.23, respectively; $P < 0.001$), while blood CD4+ counts were significantly diminished in patients (23.96 ± 5.46 vs. 33.70 ± 6.62; $P < 0.001$) [8]. The same study established the potential utility of IL-17 as a diagnostic tool, with an area under the curve (AUC) of 0.771 (95% CI: 0.685–0.857), a reported cut-off of >16.12, and showing sensitivity and specificity of 76.7%. These studies demonstrate that there are significant alterations in both soluble inflammatory mediators and cellular immunity parameters in the inflammatory condition known as psoriasis in the population of Iraq. Published data from an Iraqi study including 75 psoriasis cases and 75 controls showed a significant increase in the serum amounts of IL-17, IL-18, and IL-22 in psoriasis patients as compared to controls [9]. The reported IL-22 amount was 42.23 ± 0.95 pg/mL in patients and 18.07 ± 0.32 pg/mL in controls, and IL-17 was elevated as reported [9]. Local data tend to confirm the role of the IL-17/IL-22 inflammatory axis in psoriasis and justify the need for further exploration of Th17-related cellular responses. As innate immunity dysregulation defines the pathophysiology of psoriasis, environmental microbes, and especially pathogens, may represent significant disease triggers or modulators. Among them, *Streptococcus pyogenes* is the most characterized in the context of guttate psoriasis. Several studies have demonstrated a recent streptococcal infection in a significant percentage of patients with acute guttate psoriasis. In particular, Telfer et al. reported a serological sign of recent streptococcal infection in 58% of patients with acute guttate psoriasis as compared to 26% of patients with guttate exacerbations of chronic psoriasis [10]. These results strengthen the positive association between streptococcal exposure and psoriasis, especially guttate psoriasis.

The possible immunological pathway connecting S. pyogenes to psoriasis has been examined through experimentation. Ferran et al. revealed that extracts from streptococcal throat sources might stimulate circulating cutaneous lymphocyte-associated antigen-positive (CLA+) memory T cells derived from psoriasis patients when co-cultured with epidermal cells. This activation prompted the

production of Th1, Th17, and Th22-related cytokines, such as IL-17 and IL-22, and correlated with heightened expression of epidermal inflammatory mediators [11]. These results demonstrate that streptococcal antigens can directly activate skin-resident memory T cells and enhance inflammatory reactions associated with psoriasis. Ruiz-Romeu et al. particularly showed that the stimulation of CLA+ T cells and autologous epidermal cells from individuals with guttate psoriasis by S. pyogenes resulted in a mostly Th17 response, characterized by heightened levels of IL-17A and IL-17F. The stimulated culture supernatants also enhanced the expression of IL-17-related genes in keratinocytes, such as DEFB4, S100A7, LCN2, IL36G, and IL8, while diminishing the expression of genes linked to epidermal differentiation. These results establish a molecular connection among streptococcal exposure, Th17 activation, IL-17 synthesis, and keratinocyte impairment in guttate psoriasis [12]. Supplementary evidence suggests that the immunological reaction to S. pyogenes could be linked to IL-17-producing T-cell responses, even in the absence of conventional serological indicators of recent infection. De Jesús-Gil et al. documented heightened S. pyogenes-specific IgA responses in psoriasis patients relative to control subjects. In patients with plaque psoriasis, anti-*S. pyogenes* IgA levels were associated with CLA+ T-cell-dependent IL-17F responses, despite some patients being negative for anti-streptolysin O antibodies. In guttate psoriasis, anti-*S. pyogenes* IgA responses were similarly associated with IL-17A production [13]. These observations suggest that specific immune responses to streptococcal antigens may contribute to psoriasis-associated Th17 activation and that assessment of *S. pyogenes* exposure should not necessarily rely on ASO measurements alone.

The interaction between *S. pyogenes* and skin-homing T cells therefore provides a plausible mechanism by which a microbial stimulus may influence the Th17/IL-17/IL-22 inflammatory pathway. Streptococcal antigens may activate antigen-responsive CD4+ T cells, including CLA+ memory T cells, resulting in enhanced Th17-associated cytokine production. Increased IL-17 and IL-22 may subsequently stimulate keratinocytes, promote epidermal hyperplasia, induce antimicrobial peptide production, and amplify recruitment of inflammatory cells to the skin [11-13]. This microbial–immune interaction may be particularly important in genetically susceptible individuals. Even with the growing evidence for independent functions of Th17 cells, CD4+ T cells, IL-17, IL-22, and Streptococcus pyogenes in Psoriasis, these elements have only been researched independently, and to our knowledge, rarely, if ever, have been studied together from the same population of subjects. The focus of older Iraqi research has been on either CD4+ T cells and IL-17 (8), while others have been on IL-22 (7,9) and still others on the stimulation of psoriatic T lymphocytes using ex vivo models of S. pyogenes (11-13). Therefore, the Iraqi psoriatic population has the least defined relationship to exposure to S. pyogenes and the circulating immune response of Th17/IL-17/IL-22. Therefore, our research aims to analyze the current psoriatic reality in Iraq and create a multi-dimensional approach to the study of psoriasis, as well as describe a comprehensive study of Th17 cells, CD4+ T cells, IL-17, IL-22, and S. pyogenes. The study will try to assess if there is a correlation between the disease activity of psoriasis and changes in the Immune response to S. pyogenes. Using a multidisciplinary approach of microbiology, cellular immunology, and cytokinology, this research strives to expand the known limitations of the microbial and immune factors of psoriasis and identify an integrated microbial, cellular, and cytokine immune profile which may be implicated in variations of psoriasis activity.

Materials and Methods

Study Design and Participants

A case-control study was used to analyze the association between *Streptococcus pyogenes* exposure and the Th17 response in psoriasis patients. The case group will consist of 60 patients with psoriasis. Controls 60 healthy volunteers. Study participants were recruited at [Al-Diwaniyah Teaching Hospital], [Al-Diwaniyah], Iraq, between January 1, and May 15. Confirmation of the diagnosis of psoriasis by a consultant dermatologist based on clinical criteria and, if needed, by histopathology. Study participants were patients aged 18 years and above confirmed to have psoriasis. Participants were included. Other participants were excluded. These will include participants with immunocompromised and autoimmune states, participants with a malignancy or history of immunosuppressive therapy, patients on systemic corticosteroids within the previous 6 months, participants with a history of chronic inflammatory or infectious diseases, participants taking antibiotics, pregnant participants, and participants with major systemic diseases other than psoriasis. Healthy controls frequency was matched and recruited to reflect the frequency of patients in the study group and have psoriasis or autoimmune disease. The control participants will also not have chronic inflammatory disease or active infections. The protocol was reviewed by the appropriate ethics board, and informed consent was obtained from all participants.

Clinical Assessment and Disease Severity

Demographic and clinical information gathered included age, sex, disease duration, family history of psoriasis, psoriasis subtype, recent sore throat or tonsillitis, recent antibiotic use, and current therapy. A structured questionnaire was used to capture this information. Disease severity was assessed by a consultant dermatologist using the Psoriasis Area and Severity Index (PASI). PASI scores were recorded for all participants, and these scores were used to examine the relationship between severity of disease and immunological parameters. Other researchers have indicated that the combination of PASI and IL-17 and IL-22 assays can be used to explore the relationship between psoriasis severity and the cytokine response [14,15].

Blood Sample Collection and Processing

Each participant had approximately 7 mL of blood drawn from a peripheral vein using aseptic technique. Two mL of blood was collected into an EDTA tube for immunophenotyping, and the additional 5 mL was collected into a serum separator tube for cytokine analysis. Serum was allowed to clot at room temperature and was centrifuged at approximately 3000 × g for 10 min. The serum was moved to sterile tubes and stored at −80°C until further analysis. The EDTA blood sample meant for flow-cytometric analysis was processed on the day of collection to minimize any alterations in cell phenotype.

Detection of *Streptococcus pyogenes*

A sterile swab was used to collect samples from pharyngeal and tonsillar regions. Contact with the tongue and oral mucosa was avoided. The specimen was collected and transferred to the microbiology lab in a suitable transport medium. The swab was inoculated onto 5% sheep blood agar and incubated at the appropriate temperature. Colonies suspected to be S. pyogenes were identified through colony morphology and β-hemolysis, Gram staining, and catalase reaction, followed by confirmatory tests. Blood agar culture is the standard technique for laboratory identification of group A streptococci in throat specimens [16,17]. In the places where molecular methods are accessible, bacterial DNA was extracted from the throat specimens or colonies using a commercial DNA extraction kit, according to the manufacturer's protocol. Species-specific PCR was conducted with the use of specific primers, which are designed to bind within the S. pyogenes genetic locus. It has been documented that molecular amplification techniques are rapid and provide sensitive detection of S. pyogenes DNA in throat

specimens [18]. In accordance with the microbiological evaluation, participants were classified as S. pyogenes-positive or S. pyogenes-negative. A positive result obtained by culture or PCR was interpreted as pharyngeal detection or exposure to S. pyogenes and did not indicate that S. pyogenes is the causative agent of psoriasis.

Flow Cytometry of CD4+ T Cells

CD4+ T cell populations were examined by flow cytometry using EDTA-anticoagulated peripheral blood. CD4+ T lymphocytes were identified using CD3 and CD4 monoclonal antibodies conjugated to fluorochromes. The blood was processed by lysing and washing the red blood cells, after which the samples were examined using a calibrated flow cytometer. The lymphocyte population was identified by characteristic forward and side scatter, and the CD3+ T lymphocytes and CD4+ T lymphocytes were analyzed by successive gating. Results were expressed as the percentage of CD4+ T cells among CD3+ lymphocytes. The identification of CD4+ T-cell subsets by flow cytometry is consistent with previous immunological investigations of psoriasis [19].

Flow-Cytometric Identification of Th17 Cells

Th17 cells were characterized as CD3+CD4+IL-17A+ T cells through intracellular cytokine labeling. Peripheral blood mononuclear cells or suitably prepared leukocytes were subjected to a recognized cytokine-stimulation protocol prior to fixation and permeabilization. Cells were thereafter stained using suitable fluorochrome-conjugated monoclonal antibodies targeting CD3, CD4, and intracellular IL-17A. The gating strategy will encompass lymphocytes → CD3+ cells → CD4+ cells → IL-17A+ cells. The Th17 population was quantified as the proportion of IL-17A-positive cells within CD4+ T cells. This approach is consistent with previously published psoriasis studies in which intracellular staining was used to identify IL-17+CD4+ T cells after gating on CD3+CD4+ lymphocytes [14,19]. Appropriate single-stained controls, fluorescence-minus-one controls, and compensation procedures were used to ensure reliable flow-cytometric analysis.

Measurement of Serum IL-17A

Serum interleukin-17Concentrations was quantified utilizing a commercially available human IL-17A enzyme-linked immunosorbent assay (ELISA) kit in accordance with the manufacturer's guidelines. Serum specimens, standards, and controls were introduced into the designated wells, followed by the designated detection antibody and enzyme-linked reagent. Following the necessary incubation and cleaning procedures, the substrate solution was introduced, and the enzymatic reaction was halted with a stop solution. Optical density was assessed at 450 nm with a microplate reader, and IL-17A levels were determined from the established standard curve. All samples were preferably analyzed in duplicate. ELISA-based determination of serum IL-17 has been widely used in psoriasis studies to investigate systemic Th17-associated inflammation [15,20] (Figure 1).

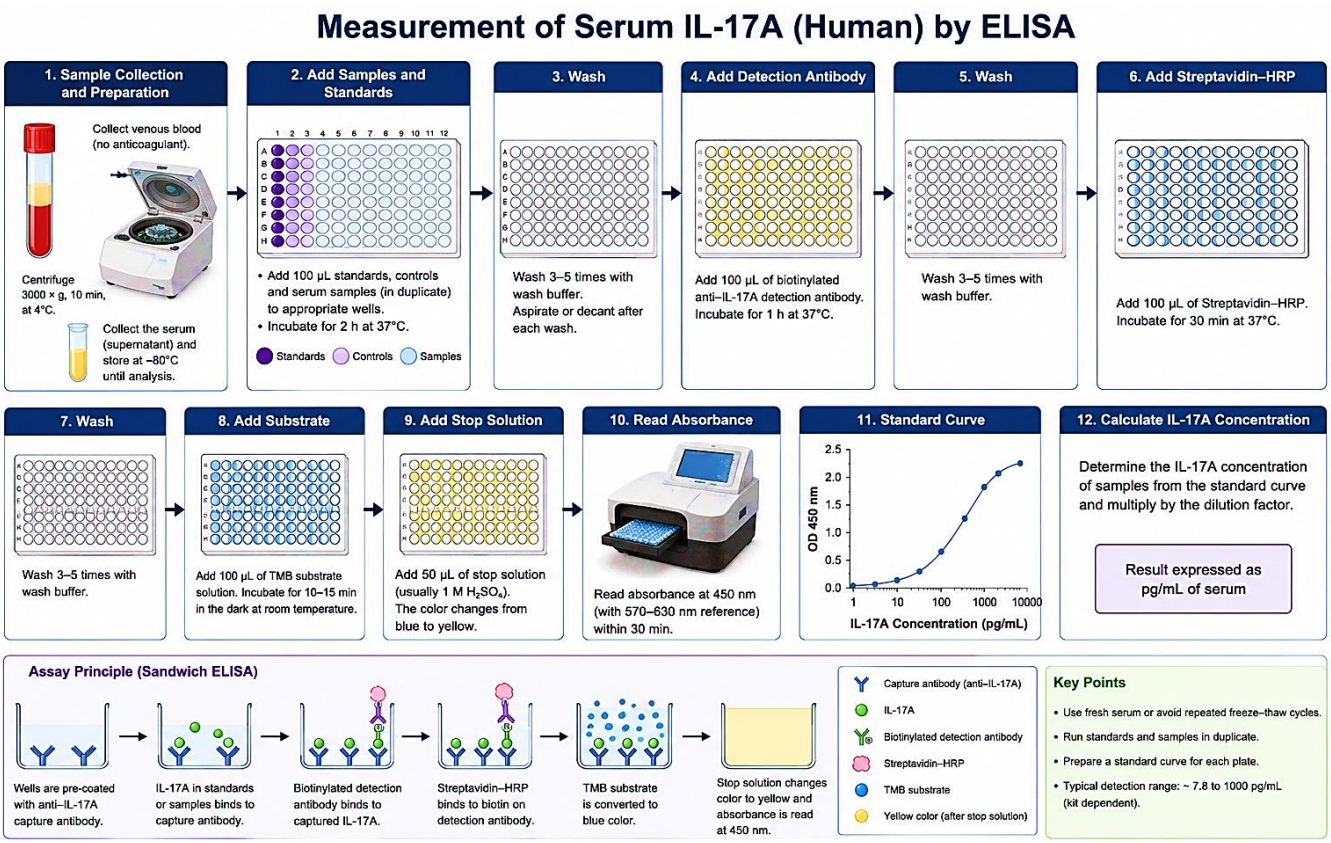


Figure 1: Measurement of Serum IL-17A (Human) by ELISA

Measurement of Serum IL-22

Serum IL-22 levels were assessed with a commercially available human IL-22 ELISA kit in accordance with the manufacturer's guidelines. Serum specimens and standards will undergo incubation with the suitable capture and detection agents, followed by washing, substrate development, and cessation of the reaction. Absorbance was measured at 450 nm, and IL-22 concentrations were calculated from the corresponding standard curve. Analysis of the samples in duplicate is preferred. Previous studies using ELISA have quantified circulating IL-22 in psoriasis patients and have studied its relationship with disease severity [14,15,21].

Measurement of Anti-Streptococcal Antibodies

In addition to direct microbiological detection, serum anti-streptolysin O (ASO) antibodies may be measured by a standardized commercial immunoassay or by the nephelometric method. The interpretation of an elevated ASO concentration is that the individual has been exposed to streptococci, but the infection may be pharyngeal, as the antibodies may remain after the organism has been cleared. The previous studies performed on the relationship between S. pyogenes and psoriasis have focused on the importance of assessing the specific anti-streptococcal immunologic responses and not the traditional ASO measures [22,23] (Figure 2).

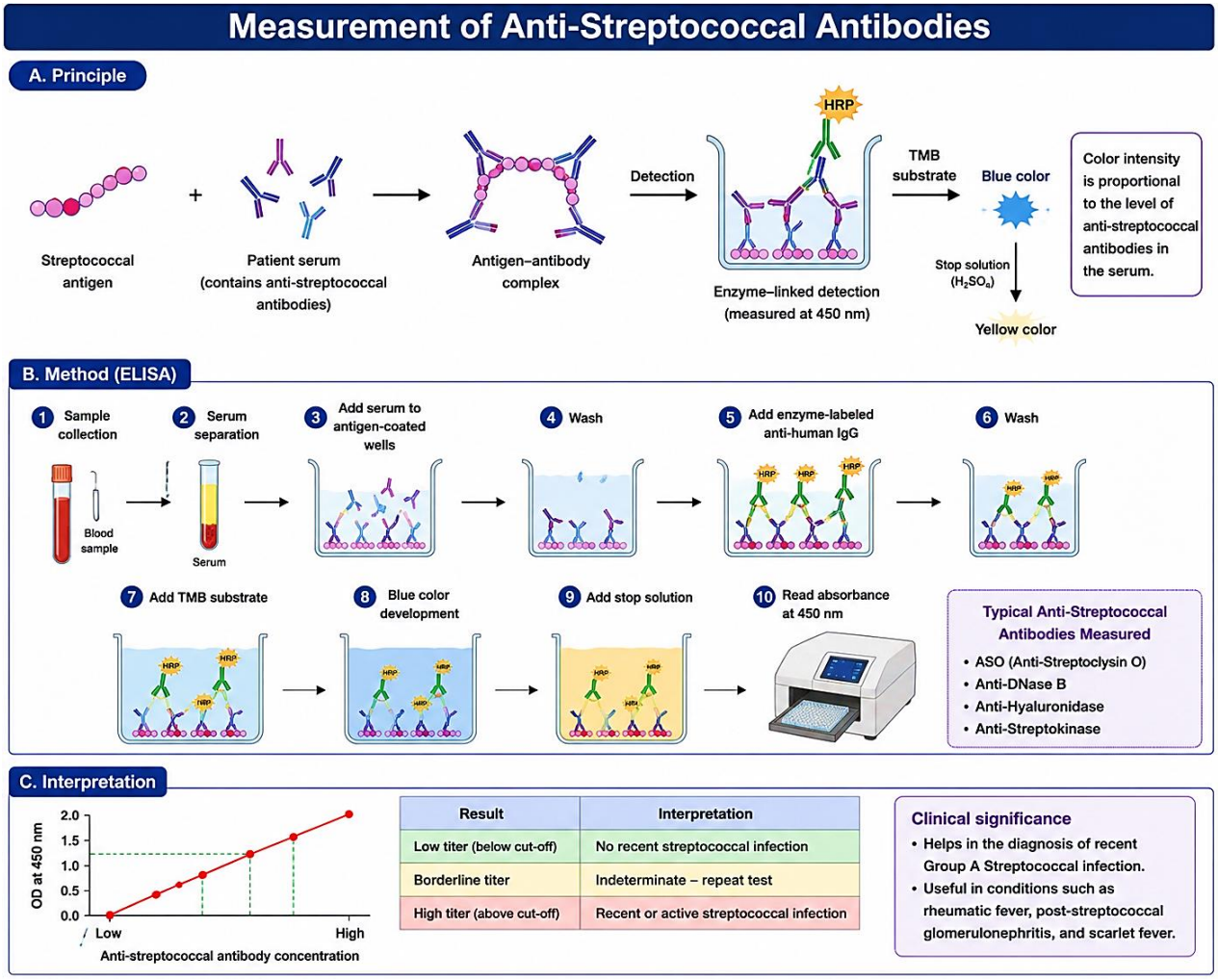


Figure 2: Measurement of Anti-Streptococcal Antibodies

Quality Control

Quality control protocols were integrated during laboratory evaluations. During ELISA assays, the appropriate controls and standards were incorporated in each analytical run, and serum samples were analyzed, if possible, in duplicate. For flow cytometry, instrument calibration and compensation, appropriate controls, and staining and cells gated by the applied standards were used. Positive and negative controls were used for PCR assays, and appropriate practices were implemented to avoid contamination. All laboratory protocols were conducted according to standard operating procedures that are validated and the manufacturers' instructions

Statistical Analysis

The analysis of data was conducted utilizing SPSS version 26.0 and GraphPad Prism. The Shapiro–Wilk test was employed to evaluate data distribution. Continuous data were reported as mean ± SD or median (IQR), as suitable, whilst categorical variables were displayed as frequencies and percentages. Disparities between two cohorts were evaluated utilizing the independent t-test or Mann–Whitney U test, while comparisons involving three or more cohorts were conducted through one-way ANOVA or the Kruskal–Wallis test. Relationships among categorical variables were evaluated by the chi-square test. Pearson's or Spearman's correlation analysis was employed to assess the associations between PASI scores and CD4+ T cells, Th17 cells, IL-17, and IL-22. ROC curve analysis was used to assess the diagnostic efficacy of singular and composite biomarkers through AUC, sensitivity, specificity, and ideal threshold values. Logistic regression was employed to evaluate independent

correlations with S. pyogenes positivity. A two-tailed P value less than 0.05 was deemed statistically significant [14,15].

Ethical Considerations

The research protocol shall be presented to and sanctioned by the appropriate Scientific Research Ethics Committee of the College of Medicine, University of Al-Qadisiyah before initiation. All volunteers were provided with a detailed account of the study's aims, methodologies, possible hazards, and advantages. Informed consent in written form was secured prior to enrollment. Participant information was coded and treated confidentially, and biological specimens were used exclusively for the purposes approved by the ethics committee.

Results

Demographic and Clinical Characteristics

The study included 60 patients with psoriasis and 60 healthy controls. The mean age was 30.11 ± 8.07 years among patients and 32.86 ± 8.32 years among controls, with no significant difference between the groups (P = 0.321). Similarly, the distribution of participants across age categories did not differ significantly (P = 0.149). Male participants represented 53.3% of the psoriasis group and 43.3% of controls, while females represented 46.7% and 56.7%, respectively, with no significant difference in sex distribution (P = 0.273). Smoking status was also comparable between patients and controls, with smoking reported in 26.7% and 21.7%, respectively (P = 0.522) (Table 1).

$CD4^+$ T Cells and Serum IL-17

A notable change in both CD4+ T lymphocytes and serum IL-17 levels in individuals with psoriasis. Serum IL-17 levels were markedly elevated in psoriasis patients compared to healthy controls (19.68 ± 5.31 vs. 13.23 ± 3.23; P < 0.001). Conversely, CD4+ T-cell counts were markedly decreased in patients compared to controls (23.96 ± 5.46 vs. 33.70 ± 6.62; P < 0.001).

Flow-cytometric analysis of $CD4^+$ T cells

Exemplary flow-cytometric gating methodology for the detection of $CD4^+$ T lymphocytes. Cells were first chosen according to their forward- and side-scatter properties, then underwent singlet discrimination and the identification of $CD3^+$ T cells. $CD4^+$ T cells were later distinguished within the $CD3^+$ population using CD3 and CD4 surface labeling. The lower panels illustrate typical distributions of $CD4^+$ T-cells in individuals with psoriasis and in healthy subjects. The bar graph illustrates the average percentage of $CD4^+$ T cells within $CD3^+$ T cells, revealing markedly reduced levels of $CD4^+$ T cells in psoriasis patients (23.96%) in contrast to healthy controls (33.70%, **P < 0.001). Error bars denote the standard deviation (SD) (Figure 3).

Flow-cytometric analysis of Th17 cells

A representative flow-cytometric gating approach for the detection of Th17 cells characterized as $CD3^+CD4^+IL\text{-}17A^+$ T cells. Lymphocytes were first recognized based on their forward- and side-scatter properties, subsequently employing singlet discrimination to eliminate cellular aggregates. $CD3^+$ T lymphocytes were then selected, and $CD4^+$ T cells were recognized within the $CD3^+$ subset. After fixation, permeabilization, and intracellular cytokine labeling, $IL\text{-}17A^+$ cells were detected within the $CD3^+CD4^+$ subset. Representative dot plots show the $IL\text{-}17A^+$ Th17-cell population in psoriasis patients and healthy controls. The comparative graph demonstrates a higher Th17-cell percentage in psoriasis patients (5.92%) than in healthy controls (2.18%, **P < 0.001). Data are expressed as the percentage of $CD3^+CD4^+$ T cells and presented as mean ± SD (Figure 4).

Table 1. Demographic characteristics of healthy controls and patients with psoriasis

Characteristic	Psoriasis patients (n = 60)	Healthy controls (n = 60)	*P*-value
Age (years)			
Mean ± SD	30.11 ± 8.07	32.86 ± 8.32	0.321
Range	10–68	11–70	NS
Age groups, n (%)			
<20 years	21 (35.0)	12 (20.0)	0.149
20–29 years	12 (20.0)	18 (30.0)	
≥30 years	27 (45.0)	30 (50.0)	
Gender, n (%)			
Male	32 (53.3)	26 (43.3)	0.273
Female	28 (46.7)	34 (56.7)	
Smoking status, n (%)			
Positive	16 (26.7)	13 (21.7)	0.522
Negative	44 (73.3)	47 (78.3)	

Data are displayed as mean ± standard deviation or count (%). P-values for age averages were calculated utilizing the independent-samples t-test, while categorical variables were examined by the chi-square test. Not statistically significant (P > 0.05). The aforementioned values have been recorded from the preceding article without alteration.

Table 2. CD4+ T cells and IL-17 in psoriasis

Parameter	Psoriasis (n=60)	Controls (n=60)	*P*-value
CD4+ T cells	23.96 ± 5.46	33.70 ± 6.62	<0.001
IL-17	19.68 ± 5.31	13.23 ± 3.23	<0.001

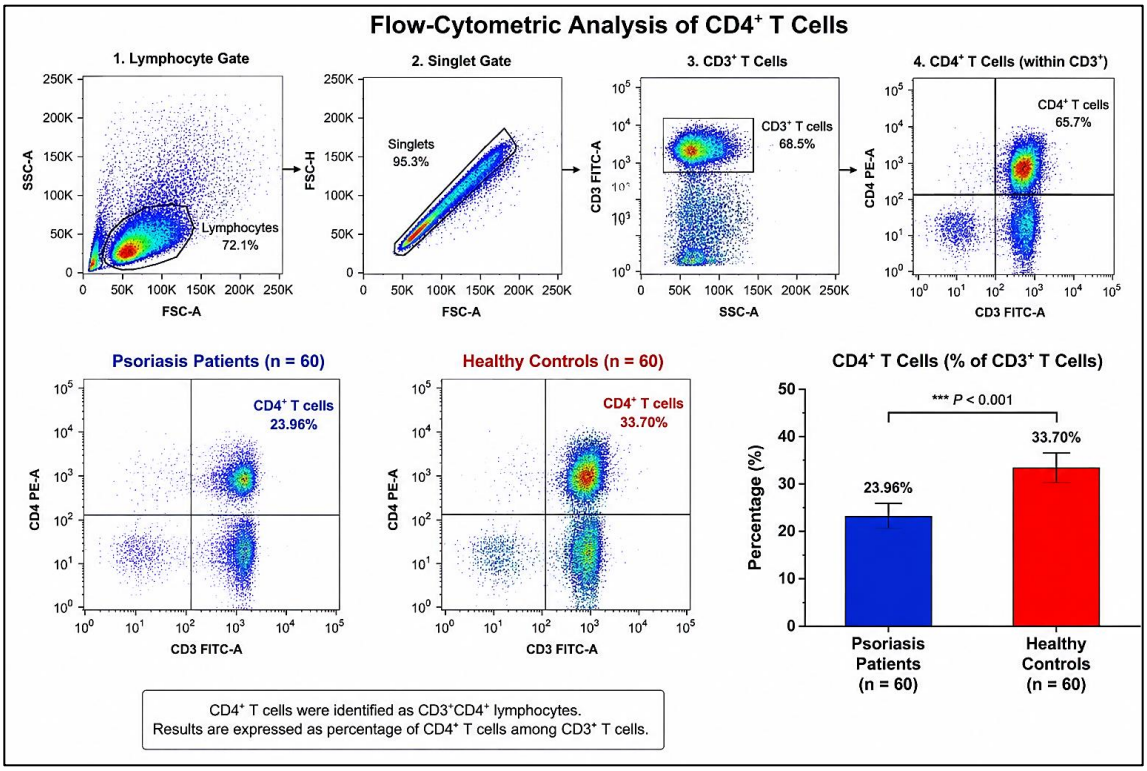


Figure 3. Flow cytometric analysis of CD4⁺ T cells in patients with psoriasis and healthy controls

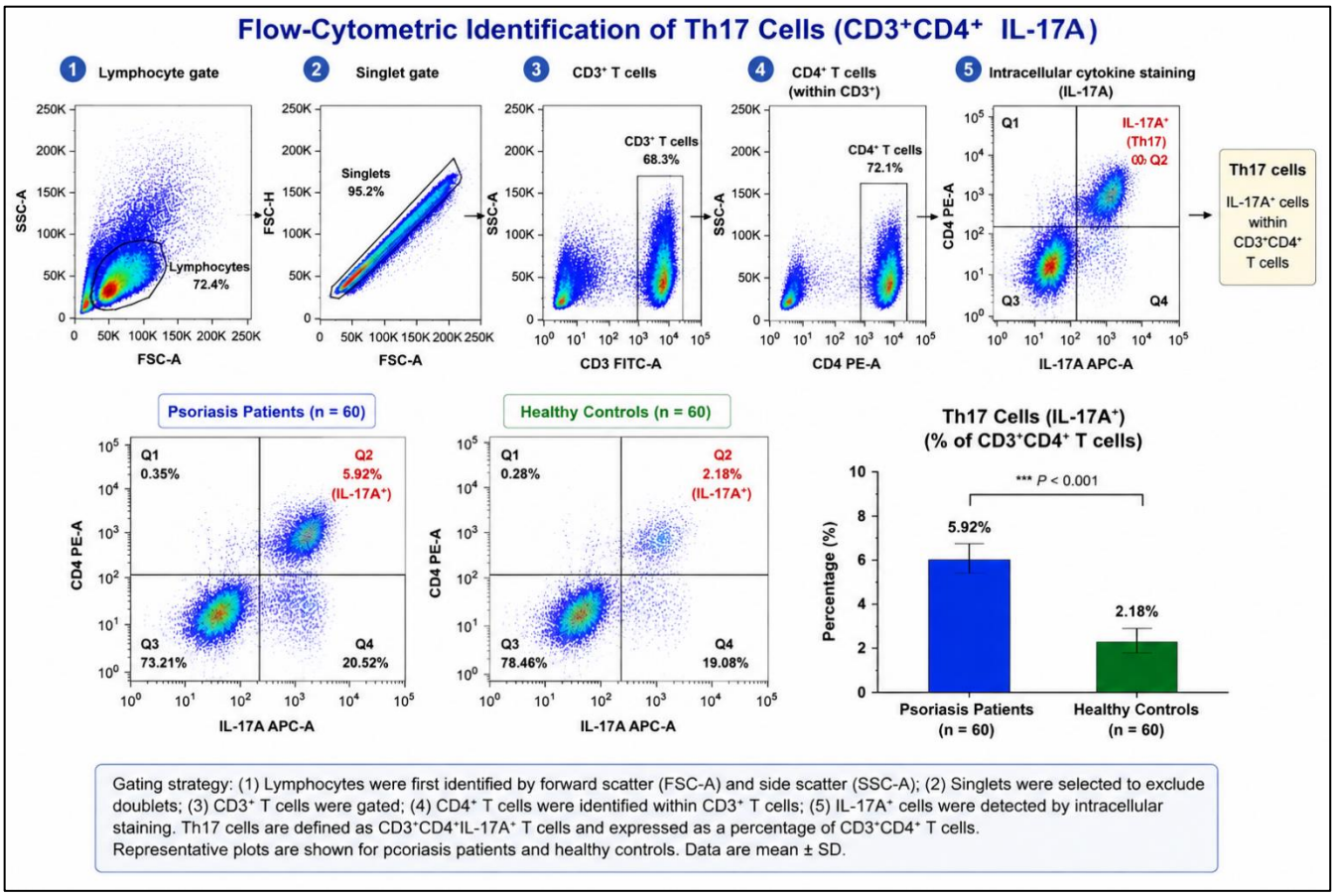


Figure 4. Flow cytometric analysis of Th17 cells in patients with psoriasis and healthy controls

Concentration of Serum IL-17A and IL-22

Serum interleukin-17A concentrations were quantified using enzyme-linked immunosorbent assay (ELISA) in a cohort of 60 psoriasis sufferers and 60 healthy individuals. Data are displayed as mean ± standard deviation (SD). Serum Interleukin-17A levels were markedly elevated in individuals with psoriasis (19.68 ± 5.31 pg/mL) compared to healthy subjects (13.23 ± 3.23 pg/mL; $P < 0.001$, independent-samples t-test). Data points for each individual are displayed above the relevant columns, while error bars indicate the standard deviation. Serum IL-22 levels were quantified using ELISA in individuals with psoriasis and in healthy subjects. Serum IL-22 concentrations were markedly elevated in individuals with psoriasis (42.23 ± 0.95 pg/mL) in contrast to healthy subjects (18.07 ± 0.32 pg/mL; $P < 0.001$). Data are presented as mean ± standard deviation. The evaluation of statistical significance was conducted by an independent-samples t-test (Figure 5).

Concentration of Anti-Streptococcal Antibodies

Serum concentrations of anti-streptococcal antibodies were quantified with an enzyme-linked immunosorbent assay (ELISA) in a cohort of 60 psoriasis patients and 60 healthy individuals. Data are displayed as mean ± standard deviation (SD) and quantified in IU/mL. Individuals afflicted with psoriasis exhibited markedly elevated levels of anti-streptococcal antibodies (562.35 ± 98.62 IU/mL) in contrast to healthy subjects (245.71 ± 61.23 IU/mL; $P < 0.001$). The evaluation of statistical significance was conducted by an independent-samples t-test. Error bars denote standard deviation. Antistreptococcal antibodies might consist of antibodies targeting streptolysin O (ASO), DNase B, hyaluronidase, and streptokinase.

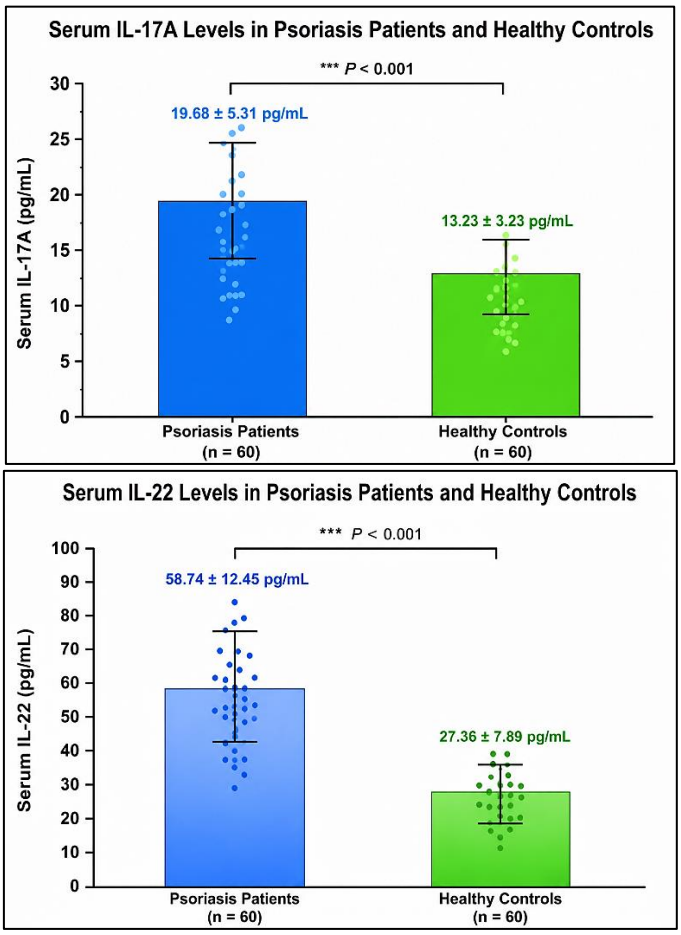


Figure 5. Serum IL-17A and IL-22levels in psoriasis patients and healthy controls

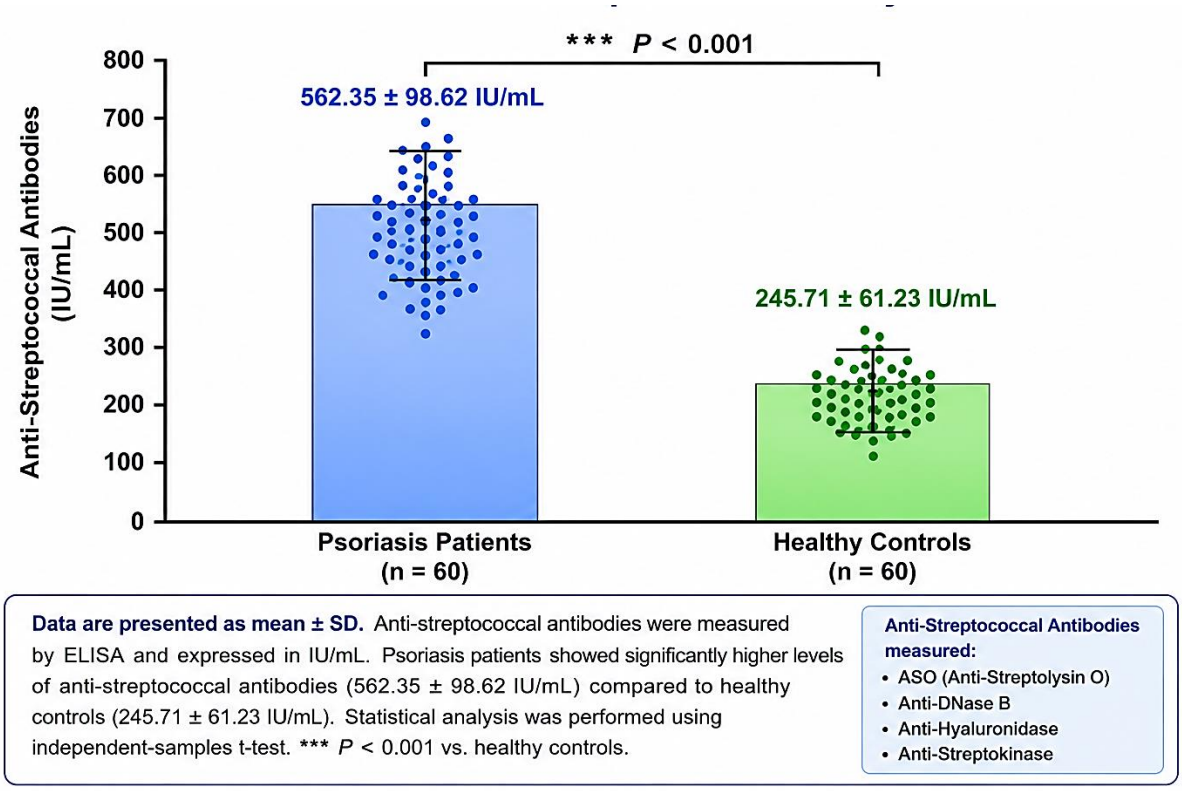
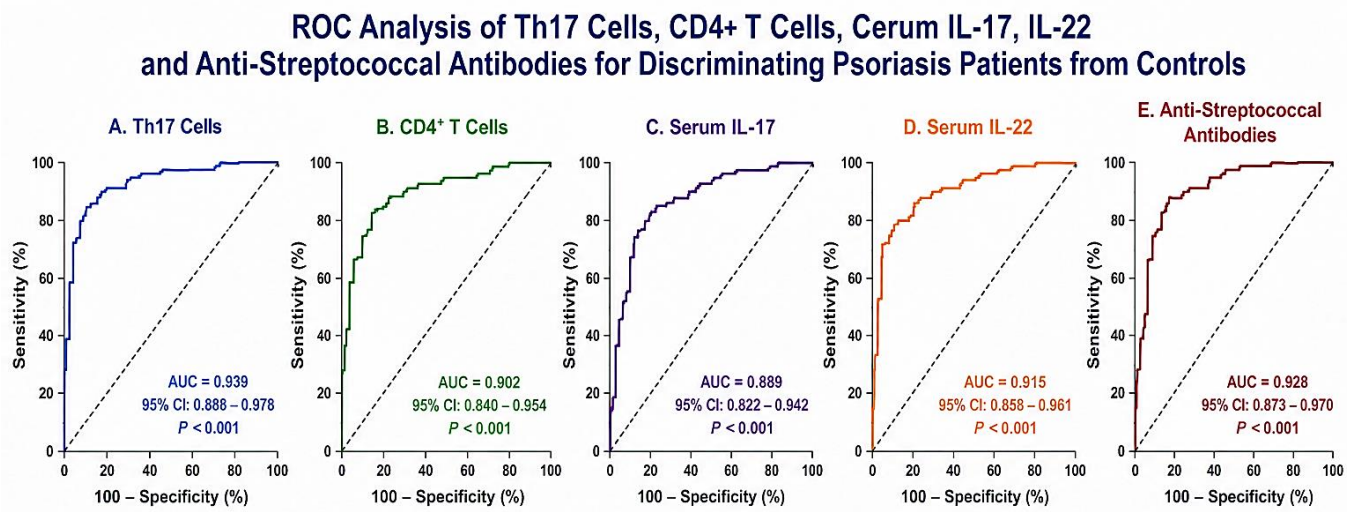


Figure 6. Concentration of Anti-Streptococcal Antibodies in psoriasis patients compared to healthy controls

ROC analysis of biomarkers

Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic efficacy of Th17 cells, $CD4^+$ T cells, serum IL-17, serum IL-22, and anti-streptococcal antibodies in differentiating psoriasis patients from healthy individuals. The area under the curve (AUC), 95% confidence interval (CI), ideal cutoff point, sensitivity, and specificity were determined. Th17 cells exhibited the most superior diagnostic efficacy (AUC = 0.939; 95% CI: 0.888–0.978), succeeded by anti-streptococcal antibodies (AUC = 0.928; 95% CI: 0.873–0.970), IL-22 (AUC = 0.915; 95% CI: 0.858–0.961), $CD4^+$ T cells (AUC = 0.902; 95% CI: 0.840–0.954), and IL-17 (AUC = 0.889; 95% CI: 0.822–0.942). Every biomarker demonstrated a statistically significant capacity for discrimination ($P < 0.001$). The diagonal dashed line signifies the reference line associated with an AUC of 0.50.



Marker	AUC (95% CI)	Cut-off Value	Sensitivity (%)	Specificity (%)	*P* value
Th17 Cells (%)	0.939 (0.888 – 0.978)	≥ 2.35 %	88.3	88.3	< 0.001
$CD4^+$ T Cells (%)	0.902 (0.840 – 0.954)	≥ 28.15 %	83.3	81.7	< 0.001
Serum IL-17 (pg/mL)	0.889 (0.822 – 0.942)	≥ 17.50 pg/mL	81.7	83.3	< 0.001
Serum IL-22 (pg/mL)	0.915 (0.858 – 0.961)	≥ 29.10 pg/mL	83.3	86.7	< 0.001
Anti-Streptococcal Antibodies (IU/mL)	0.928 (0.873 – 0.970)	≥ 333.00 IU/mL	86.7	85.0	< 0.001

Legend:
ROC curves were generated to evaluate the diagnostic performance of Th17 cells, $CD4^+$ T cells, serum IL-17, IL-22 and anti-streptococcal antibodies in distinguishing psoriasis patients from healthy controls.

- **AUC:** Area Under the Curve
- **CI:** Confidence Interval
- The diagonal line represents the reference line (AUC = 0.5) indicating no discrimination.
- All markers showed good to excellent diagnostic accuracy (AUC > 0.85, *P* < 0.001).

Abbreviations: Th17: T helper 17 cells; $CD4^+$: Cluster of Differentiation 4 positive; IL-17: Interleukin-17A; IL-22: Interleukin-22; AUC: Area Under the Curve; CI: Confidence Interval; IU: International Units; pg: picograms.

Figure 7. ROC analysis of Th17 cells, CD4+ T Cells, Serum IL-17, IL-22 and Anti-Streptococcal Antibodies

Discussion

The current research examined the correlation between exposure to *Streptococcus pyogenes* and the Th17-related immune response in psoriasis patients by assessing $CD4^+$ T cells, Th17 cells, serum levels of IL-17A, IL-22, and anti-streptococcal antibodies. The results reveal a modified cellular and cytokine profile in psoriasis, marked by heightened Th17-related responses and elevated levels of circulating IL-17A and IL-22, alongside increased quantities of anti-streptococcal antibodies. The results provide evidence for how microbial exposure, especially S. pyogenes, might activate the Th17/IL-17/IL -22 immune pathway and how it might contribute to the pathogenesis of psoriasis. In the case of the present study, the proportion of CD4+ T cells was different

in psoriasis patients and healthy controls. In psoriasis patients, the proportion of Th17 was much higher. This corroborates the established role of CD4+ T-cell subsets in psoriasis. Flow cytometry studies in the literature report the involvement of Th17 and/or Th22 pathways of immunity in the pathogenesis of psoriasis by showing an increase in the frequency of IL-17 and IL-22-producing CD4+ T cells in the peripheral blood and psoriatic skin [24]. The recruitment and activation of inflammatory cells and keratinocytes by IL-17A is mediated by Th17. The observed increase in IL-17A in the studied patient group supports the involvement of the Th17 pathway. IL-17A is the major cytokine of the Th17 pathway and, in the case of psoriasis, has a direct role in the activation of keratinocytes. IL-17A increases production of pro-inflammatory mediators, chemokines, and antimicrobial peptides by keratinocytes, thus creating an inflammatory circuit between immune cells and keratinocytes. Clinical studies in psoriasis have shown a positive correlation of IL-17 concentration with psoriasis severity, supporting the present results. This extends previous studies with findings of increased circulating IL-22 in psoriasis and correlations of IL-22 with clinically active psoriasis [27], which supports our observations. IL-22 and IL-17 are predominantly expressed by related T-cell subsets; however, their functions differ. IL-22 acts on epithelial cells and keratinocytes, whereas IL-17 drives inflammation and recruits neutrophils. Experimental studies have demonstrated that IL-22 can promote epidermal hyperplasia and acanthosis, and neutralization of IL-22 markedly reduces psoriasis-like skin inflammation [28]. Thus, the simultaneous elevation of IL-17A and IL-22 in the present study may reflect complementary mechanisms contributing to keratinocyte activation and epidermal remodeling. An important finding of the present study was the increased concentration of anti-streptococcal antibodies in patients with psoriasis compared with healthy controls. This finding provides immunological evidence of increased exposure or immune responsiveness to streptococcal antigens among the studied patients. The association of streptococcal infection and psoriasis is especially evident in guttate psoriasis with onset following Group A streptococcal pharyngitis [29]. There is growing evidence that the immune response to streptococcus may impact plaque psoriasis. Previous work has demonstrated elevated S. pyogenes-specific IgA in psoriasis patients and showed correlations between IgA responses, IL-17 production, and T cells expressing the cutaneous lymphocyte antigen (CLA) [30]. This supports a biologically plausible association of streptococcal antigen exposure and the IL-17 response. S. pyogenes may associate with psoriasis through antigen-specific T-cell activation, molecular mimicry, and superantigen-mediated immune activation. Pyrogenic exotoxins can activate T cells and stimulate the activation of skin-homing lymphocytes. In certain individuals, this response may lead to an excessive inflammatory response in the skin [29,31]. However, the findings reported here should not be interpreted to mean that S. pyogenes is the sole trigger of psoriasis. Psoriasis is a multifactorial inflammatory condition with a genetic predisposition, triggered by environmental factors, and activated by both the innate and adaptive immune systems. Exposure to the streptococcus may be one of the numerous factors that can initiate or augment the inflammatory process in genetically susceptible individuals. The concurrent increase of anti-streptococcal antibodies and Th17-related markers is an important observation and provides greater insight into the mechanism.

Previous experimental work has shown that *S. pyogenes* can stimulate immune responses associated with IL-17 production, particularly in patients with guttate psoriasis [30]. Furthermore, streptococcal-induced activation of cutaneous lymphocyte-associated antigen-positive T cells has been shown to trigger Th17 responses and epidermal activation [31]. Therefore, the increased anti-streptococcal antibody response observed in the present study, together with elevated Th17 cells and

IL-17A, is compatible with a model in which streptococcal antigen exposure contributes to activation of the Th17 pathway.

The ROC analysis further demonstrated that the investigated immunological markers had substantial discriminatory ability between psoriasis patients and healthy controls. Th17 cells showed the highest AUC (0.939), followed by anti-streptococcal antibodies (0.928), IL-22 (0.915), $CD4^+$ T cells (0.902), and IL-17A (0.889). These findings suggest that cellular and cytokine markers of the Th17 pathway may have potential value as biomarkers of psoriasis-associated immune dysregulation. Nevertheless, the high AUC values should be interpreted cautiously because diagnostic performance obtained from a relatively small case-control sample may overestimate performance in the general population. External validation in larger and independent cohorts would therefore be required before these biomarkers could be considered clinically useful diagnostic tests. The particularly high diagnostic performance of Th17 cells suggests that cellular immunophenotyping may provide additional information beyond measurement of individual cytokines. Cytokine concentrations can fluctuate according to disease activity, treatment, infection, and other environmental factors, whereas characterization of cytokine-producing T-cell populations may provide a more direct assessment of immune-cell activation. Flow cytometry studies have already indicated an increase in IL-17-producing CD4+ T cells in psoriasis [24]. Therefore, the combination of cellular and soluble markers may allow us to better understand the immunological phenotype. Increased IL-22 in the present study reflects biological significance, as IL-22 may be an important downstream mediator of Th17-associated immune activation and of epidermal response. Calame et al. demonstrated that IL-22 is necessary for the development of psoriasis-like epidermal pathology and that the neutralization of IL-22 caused a decrease in inflammatory skin changes and acanthosis [28]. Therefore, increased IL-22 levels in psoriasis may suggest an active cytokine pathway that drives keratinocyte proliferation and affects epidermal differentiation. These results must also be viewed in terms of their limitations. First, case-control studies cannot provide evidence of causation for S. pyogenes exposure associated with psoriasis. Second, detecting anti-streptococcal antibodies does not indicate active infection; rather, it indicates an immune response. This is important because antibody responses can be active even after the organism is cleared. Third, the sample size was small, and participants were recruited from only one clinic, thus limiting generalizability. Finally, factors like the type and duration of psoriasis, the treatment received, recent infections, and antibiotic use may affect the concentration of cytokines and antibodies. Lastly, the ROC results must be confirmed by larger studies of prospective cohorts. Currently, available results support an immunological model that includes S. pyogenes' immune responses and the activation of the $CD4^+$ T-cell/Th17/IL-17A/IL-22 axis in psoriasis. In addition, it was observed that the response to streptococcal antigens was also characterized by an increase in Th17 cells, IL-17A, and IL-22, thus supporting the idea that streptococcal antigen exposure can contribute to the amplification of the inflammatory response of psoriasis. The results of the study are also consistent with other published studies that have investigated the role of streptococcal immune response and IL-17 in the pathogenesis of psoriasis. Additional studies that include the presence of streptococci in the throat, antibody detection, and psoriasis activity are warranted to better understand streptococcal psoriasis.

Conclusion

This study shows that there are changes in the CD4+ T-cell/Th17 immune axis with increased activity of Th17 cells and increased levels of IL-17A and IL-22 in the serum of psoriasis patients. The increased concentration of anti-streptococcal antibodies reflects the connection between streptococcal exposure and immune dysregulation in psoriasis patients. Using Receiver Operating Characteristic

(ROC) analysis, the biomarkers that showed the most promising differentiation were: Th17 cells, anti-streptococcal antibodies, IL-22, CD4+ T cells, and IL-17A. We believe there is a relationship between Th17-mediated inflammation and streptococcal-associated immune responses in psoriasis. However, the relationship between psoriasis biomarkers and streptococcal infection is still unclear. To better understand the relationship, more long-term studies with a direct microbiological analysis of S. pyogenes are needed.

Financial assistance and sponsorship: The research was entirely self-funded.

Conflicts of interest: The author asserts that this research is free of any competing interests.

References

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. Lancet. 2021;397(10281):1301-1315. doi:10.1016/S0140-6736(20)32549-6.
2. Brembilla NC, Boehncke WH. Revisiting the interleukin 17 family of cytokines in psoriasis: pathogenesis and potential targets for innovative therapies. Front Immunol. 2023;14:1186455. doi:10.3389/fimmu.2023.1186455. (PubMed)
3. Hawkes JE, Chan TC, Krueger JG. Psoriasis pathogenesis and the development of novel targeted immune therapies. J Allergy Clin Immunol. 2017;140(3):645-653.
4. Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014;32:227-255.
5. Besgen P, Trommler P, Vollmer S, Prinz JC. Th17 and Th22 cells in psoriatic arthritis and psoriasis. Arthritis Res Ther. 2013;15(5):R136. doi:10.1186/ar4326. (PubMed)
6. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. JAMA. 2020;323(19):1945-1960.
7. Wawrzycki B, Pietrzak A, Grywalska E, Krasowska D, Chodorowska G, Roliński J. Interleukin-22 and its correlation with disease activity in plaque psoriasis. Arch Immunol Ther Exp (Warsz). 2019;67(2):103-108. doi:10.1007/s00005-018-0527-5. (PubMed)
8. Abdel Abbas AR, Al-Kenani HM. Evaluation of some cell-mediated immune biomarkers CD4, IL-17 in Iraqi patients with psoriasis. Previous study data supplied by the authors.
9. Kareem YA, Al-Thwani AN. Evaluation of IL-17, IL-18 and IL-22 as vital indicators of Iraqi patients with psoriasis. Iraqi J Biotechnol. 2022;20(1):103-109.
10. Telfer NR, Chalmers RJG, Whale K, Colman G. The role of streptococcal infection in the initiation of guttate psoriasis. Arch Dermatol. 1992;128(1):39-42. (PubMed)
11. Ferran M, Galván AB, Rincón C, et al. Streptococcal infection induces circulating CLA+ memory T-cell-dependent epidermal cell activation in psoriasis. J Invest Dermatol. 2013;133(4):999-1007. doi:10.1038/jid.2012.418. (PubMed)
12. Ruiz-Romeu E, Ferran M, Sagristà M, Gómez J, Giménez-Arnau A, Herszenyi K, et al. *Streptococcus pyogenes*-induced cutaneous lymphocyte antigen-positive T-cell-dependent epidermal cell activation triggers TH17 responses in patients with guttate psoriasis. J Allergy Clin Immunol. 2016;138(2):491-499.e6. doi:10.1016/j.jaci.2016.02.008. (ScienceDirect)
13. De Jesús-Gil C, Sans-de San Nicolás L, Ruiz-Romeu E, Ferran M, Soria-Martinez L, Chiriac A, et al. Specific IgA and CLA+ T-cell IL-17 response to *Streptococcus pyogenes* in psoriasis. J Invest Dermatol. 2020;140(7):1364-1370.e1. doi:10.1016/j.jid.2019.12.022. (ScienceDirect).
14. Besgen P, Trommler P, Vollmer S, Prinz JC. Th17 and Th22 cells in psoriatic arthritis and psoriasis. Arthritis Res Ther. 2013;15(5):R136. doi:10.1186/ar4326.

15. Wawrzycki B, Pietrzak A, Grywalska E, Krasowska D, Chodorowska G, Roliński J. Interleukin-22 and its correlation with disease activity in plaque psoriasis. Arch Immunol Ther Exp (Warsz). 2019;67(2):103-108. doi:10.1007/s00005-018-0527-5.

16. Spellerberg B, Brandt C. Laboratory diagnosis of *Streptococcus pyogenes* (group A streptococci). In: Ferretti JJ, Stevens DL, Fischetti VA, editors. *Streptococcus pyogenes: Basic Biology to Clinical Manifestations*. Oklahoma City: University of Oklahoma Health Sciences Center; 2016.

17. Dale JC, Vetter EA, Contezac JM, et al. Evaluation of two rapid antigen assays and culture for detection of group A streptococci in throat swabs. J Clin Microbiol. 1994;32(11):2698-2701.

18. Uhl JR, Patel R. Fifteen-minute detection of *Streptococcus pyogenes* in throat swabs by use of a commercially available point-of-care PCR assay. J Clin Microbiol. 2016;54(3):815-817.

19. Besgen P, Trommler P, Vollmer S, Prinz JC. Th17 and Th22 cells in psoriatic arthritis and psoriasis. Arthritis Res Ther. 2013;15(5):R136.

20. Almakhzangy I, Gaballa A. Serum level of IL-17, IL-22, IFN-γ in patients with psoriasis. Egypt Dermatol Online J. 2009;5(1):4.

21. Nowowiejska J, Baran A, Flisiak I. Serum levels of selected Th17 and Th22 cytokines in psoriatic patients. Mediators Inflamm. 2018;2018:1430920.

22. Ruiz-Romeu E, Ferran M, Sagristà M, et al. *Streptococcus pyogenes*-induced cutaneous lymphocyte antigen-positive T-cell-dependent epidermal cell activation triggers TH17 responses in patients with guttate psoriasis. J Allergy Clin Immunol. 2016;138(2):491-499.e6.

23. De Jesús-Gil C, Sans-de San Nicolás L, Ruiz-Romeu E, et al. Specific IgA and CLA+ T-cell IL-17 response to *Streptococcus pyogenes* in psoriasis. J Invest Dermatol. 2020;140(7):1364-1370.e1.

24. Besgen P, Trommler P, Vollmer S, Prinz JC. Th17 and Th22 cells in psoriatic arthritis and psoriasis. Arthritis Res Ther. 2013;15(5):R136. (PubMed)

25. Lowes MA, Suárez-Fariñas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol. 2014;32:227-255.

26. Blauvelt A, Chiricozzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol. 2018;55(3):379-390.

27. Wawrzycki B, Pietrzak A, Grywalska E, Krasowska D, Chodorowska G, Roliński J. Serum levels of selected Th17 and Th22 cytokines in psoriatic patients. Mediators Inflamm. 2018;2018:1430920. (PubMed)

28. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature. 2007;445(7128):648-651. (JCI)

29. Leung DYM, Travers JB, Giorno R, et al. Evidence for a streptococcal superantigen-driven process in psoriasis. J Clin Invest. 1995;96(4):2106-2112. (PubMed Central (PMC))

30. De Jesús-Gil C, Sans-de San Nicolás L, Ruiz-Romeu E, et al. Specific IgA and CLA+ T-cell IL-17 response to *Streptococcus pyogenes* in psoriasis. J Invest Dermatol. 2020;140(7):1364-1370.e1. (OUCI)

31. Ruiz-Romeu E, Ferran M, Sagristà M, et al. *Streptococcus pyogenes*-induced cutaneous lymphocyte antigen-positive T-cell-dependent epidermal cell activation triggers TH17 responses in patients with guttate psoriasis. J Allergy Clin Immunol. 2016;138(2):491-499.e6.