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The Diagnostic Discriminative Power of Serum IL-6 Levels for Chlamydia Trachomatis Infection

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Abstract

Background: *Chlamydia trachomatis* represents one of the most prevalent sexually transmitted infections, frequently persisting without noticeable symptoms, yet capable of leading to adverse outcomes such as infertility. The identification of reliable biomarkers has been emphasized as essential for enhancing early detection strategies. **Objective:** This research was conducted to evaluate the potential utility of circulating interleukin-6 (IL-6) as a biomarker for the diagnosis of *Chlamydia trachomatis* infection. **Methods:** A case–control approach was adopted, comprising 52 individuals with laboratory-confirmed *C. trachomatis* infection and 48 apparently healthy participants serving as controls. Serum IL-6 levels were quantified using the enzyme-linked immunosorbent assay (ELISA). Group differences were examined through independent samples t-tests, while variations associated with different infection sites were assessed using analysis of variance (ANOVA). Additionally, the diagnostic performance of IL-6 was evaluated via receiver operating characteristic (ROC) curve analysis to determine its accuracy in distinguishing infected from non-infected subjects. **Results:** Patients had significantly elevated IL-6 levels (18.6 ± 5.2 pg/ml) compared to controls (12.4 ± 4.1 pg/ml, $p < 0.03$). Analysis of serum IL-6 levels across different anatomical infection sites indicated that concentrations were highest in urogenital infections (19.2 ± 5.1 pg/ml), whereas rectal infections exhibited moderately lower values (17.5 ± 4.6 pg/ml). The area under the curve (AUC) was 0.84, with an optimal diagnostic threshold determined at 15.5 pg/ml, yielding a sensitivity of 80.8% and specificity of 77.1%. These findings suggest that IL-6 levels are markedly elevated in individuals affected by *Chlamydia trachomatis*, supporting its potential role as a reliable biomarker, particularly for urogenital infection sites.

Keywords: Interleukin-6, Chlamydia trachomatis, AUC, Sensitivity, Specificity

Introduction

Sexually transmitted diseases (STDs) exhibited a great worldwide health challenge with far-reaching social, economic, and public health implications. These infections have elicited humanity for many decades, spreading out of geographical limits and social contexts, with Chlamydia trachomatis as the most common bacterial causative agents. In 2020, WHO estimated 374 million new infections with 1 of 4 STIs: chlamydia (129 million), gonorrhoea (82 million), syphilis (7.1 million) and trichomoniasis (156 million). It was estimated that 128.5 million new infections occurred among adults aged 15–49 years globally. Many of these infections are asymptomatic, particularly in women, which hampers early detection and treatment and leads to complications such as pelvic inflammatory disease, infertility, ectopic pregnancies, and adverse pregnancy outcomes (World Health Organization, 2023).

Timely diagnosis of *C. trachomatis* infection is essential to preventing these sequelae. The gold standard diagnostic methods are nucleic acid amplification tests (NAATs), which have high sensitivity and specificity.

However, NAATs require laboratory infrastructure, trained technologists, and may entail logistic delays and costs that preclude their widespread use in low-resource settings or in screening large populations. Therefore, there is considerable interest in identifying biomarkers that are less expensive, easier to deploy, and capable of discriminating infected from uninfected individuals, including in early or asymptomatic stages of disease.

The immune response to infection involves a variety of cytokines and chemokines; among them, interleukin-6 (IL-6) has emerged as a cytokine of particular interest. IL-6 is a multifunctional cytokine, formed by many cell types (including epithelial cells, fibroblasts, macrophages, and monocytes) in response to infection, tissue injury, or inflammation. It participates in the acute phase response, stimulates B and T cell activation, influences differentiation of immune cell subsets, and orchestrates transitions between innate and adaptive immunity (Sun et al., 2017). Because of its early induction and systemic effects, IL-6 has been explored in many infectious diseases as both a prognostic marker (e.g., severity) and potentially diagnostic tool.

Several studies have demonstrated that IL-6 levels rise in response to bacterial infections. For example, Dalrymple, et al. (1996) showed that IL-6–deficient mice are more susceptible to systemic *Escherichia coli* infection, with significantly higher bacterial loads and mortality compared to controls, indicating that IL-6 plays a protective role in host defense. Moreover, murine models of gram-negative bacterial challenge show rapid IL-6 induction both at mucosal surfaces and in serum, with levels correlating to infection intensity (de Man et al., 1989).

Turning specifically to *Chlamydia trachomatis*, a body of research is now elucidating how IL-6 is involved in the local immune response. In cell culture studies, epithelial cells or co-cultures of epithelial and immune cells infected with *C. trachomatis* exhibit increased IL-6 secretion. For instance, a study using HeLa epithelial cells and monocyte-like THP-1 cells found that IL-6 (along with IL-8) secretion was sustained following infection, particularly in co-culture settings, indicating persistent inflammatory signaling even when infection is under partial control or during antibiotic-induced persistence (Mpiga et al., 2006). Similarly, primary reproductive epithelial cultures and peripheral blood mononuclear cells (PBMCs) from human donors show variable but detectable IL-6 responses when stimulated with live *C. trachomatis* or chlamydial stress-response antigens (e.g. HtrA, Tsp) (Russell et al., 2014).

In spite these insights, there still a shortage in the literature with respect to whether serum IL-6 levels (i.e. systemically circulating IL-6) can reliably discriminate individuals with *C. trachomatis* infection from those without. Key questions include: What are the sensitivity and specificity of serum IL-6? How early in the course of infection does it rise to detectable levels? What are the baseline levels among uninfected populations, and how much overlap exists? Are there host factors (age, co-infections, immune status) that affect IL-6 levels independently of infection status?

The diagnostic discriminative power of IL-6, if adequate, could offer important adjunctive utility: potentially quicker screening, triage in clinical settings, or even point-of-care testing in resource-limited environments. In addition, serum biomarkers can be especially helpful when anatomical sampling is difficult, or when patient discomfort or stigma reduces uptake of genital sampling. Therefore, this study aims evaluate the discriminative power of serum IL-6 levels for diagnosis of *Chlamydia trachomatis* infection. We aim to (a) compare serum IL-6 concentrations in infected versus uninfected individuals, (b) assess how well serum IL-6 discriminates infection status via measures such as sensitivity, specificity, and receiver operating characteristic (ROC) curve analysis, and (c) consider whether serum IL-6 could serve as a practical biomarker in routine care or in screening programs. The findings may help clarify whether IL-6 can supplement existing diagnostic methods, especially in settings where molecular diagnostics are less available.

Patients and Methods

Study Design and Setting

This case–control study was conducted at Hussein Teaching Hospital in Karbala, Iraq, between August 2024 and February 2025. The hospital is a tertiary referral center that serves Karbala and neighboring provinces, providing access to a diverse patient population.

Study Population

A total of 52 patients with confirmed *Chlamydia trachomatis* infection were included. Patients were aged between 22 and 45 years and included both men and women. Diagnosis of *C. trachomatis* infection was established using nucleic acid amplification tests (NAATs) performed on endocervical swabs (for women) or urethral swabs/urine samples (for men), following

Centers for Disease Control and Prevention (CDC) recommendations. The healthy group (control) included of 48 apparently healthy volunteers recruited from hospital staff and blood donors, with no prior history of STIs or chronic inflammatory conditions.

Inclusion and Exclusion Criteria

Patients were eligible if they had a confirmed diagnosis of *C. trachomatis* infection during the study period. Exclusion criteria were:

- Presence of chronic systemic diseases such as malignancy, or chronic kidney disease, autoimmune disease, cardiovascular disease, diabetes mellitus.
- Current or recent (within one month) antimicrobial or anti-inflammatory therapy.
- Co-infection such as HIV, syphilis, gonorrhea.
- Pregnancy or lactation.

Controls were included only if they had negative results for *C. trachomatis* by NAAT, and no clinical signs of acute or chronic infections. These criteria were established to ensure that the measured serum IL-6 levels specifically reflected the immunological response to *C. trachomatis* infection.

Clinical and Laboratory Examination

All patients were subjected to medical history assessment and clinical examination. Demographic data, risk factors, and clinical symptoms (e.g., dysuria, vaginal/urethral discharge, pelvic or testicular pain) were recorded. Routine laboratory investigations included C-reactive protein (CRP) and total WBC count to assess systemic inflammation.

Sample Collection and Processing

For cytokine measurement, 3 ml of venous blood was obtained from each participant using sterile technique. Blood samples were collected into plain vacutainers and allowed to clot at room temperature. Samples were subjected to centrifugation at 3000 rpm for 10 minutes to isolate serum, which was aliquoted into sterile Eppendorf tubes and stored at –20 °C until analysis.

Measurement of Serum IL-6

Serum interleukin-6 (IL-6) levels have been quantified using a enzyme-linked immunosorbent assay (ELISA) kit (e.g., Germany, Humacount). The assay was implemented based on the manufacturer's leaflet. Briefly, standards and serum samples were pipetted into microtiter wells pre-coated with anti-human IL-6 antibody. After incubation and washing, streptavidin-horseradish peroxidase (HRP) and a biotin-conjugated secondary antibody were added. Color development was achieved with a substrate solution, and the reaction was stopped with 2N sulfuric acid. All samples were run in duplicate, and internal quality control sera were included in each run to ensure assay reproducibility and accuracy.

Statistical Analysis

Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation (SD). Comparisons between patient and control groups were conducted using the independent samples t-test for quantitative data and the chi-square (χ^2) test for qualitative data. Receiver operating characteristic (ROC) curve analysis has been performed to test the diagnostic discriminative power of serum IL-6 levels, with sensitivity, specificity. A p-value < 0.05 was considered statistically significant for all tests. Multiple comparison between groups were done by f teat and LSD (Al-Fahham, 2018).

Results

The demographic distribution of the studied groups (patients and controls) demonstrated comparable characteristics with respect to age, sex, and smoking habits. The majority of participants were within the 21–40-year age range, reflecting the sexually active population most at risk for *Chlamydia trachomatis* infection. The male-to-female ratio was nearly balanced between the two groups, indicating no sex-based sampling bias. Furthermore, smoking habits did not differ significantly between patients and controls, suggesting that smoking was unlikely to act as a confounding factor in the present analysis. Overall, the non-significant chi-square values across these variables confirm that the case and control groups were appropriately matched, thereby strengthening the reliability of subsequent comparisons in relation to biomarker levels (Table 1).

Table 1. Age, sex and smoking habit distribution of investigated subjects with Chlamydia trachomatis infection

Indicators		`Patients (No. = 52)		Control (No. = 48)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	21-30	20	38.50	18	37.50		0.45 (NS)
	31-40	17	32.70	15	31.30		
	41-50	10	19.2	9	18.8		
	> 50	5	9.6	6	12.5		
Sex	Male	28	53.8	24	50		0.53 (NS)
	Female	24	46.2	24	50		
Smoking	Smoker	18	34.6	14	29.2		0.11 (NS)
	Non-smoker	34	65.4	34	70.8		

NS: Non-significant at P>0.05

The distribution of *Chlamydia trachomatis* infections in the study population revealed that the urogenital site was the most frequently affected, with 36 cases (69.2%). In comparison, rectal infections accounted for 8 cases (15.4%), pharyngeal infections for 5 cases (9.6%), and conjunctival involvement for only 3 cases (5.8%). This distribution aligns with the well-documented epidemiology of C. trachomatis, where urogenital transmission represents the dominant route of infection. The lower frequencies of extragenital sites may reflect variations in exposure risk, sexual practices, and the often-asymptomatic nature of such infections, which contributes to underdiagnosis. These findings underscore the importance of prioritizing urogenital screening in clinical practice while also considering extragenital testing to prevent persistent, undetected reservoirs of infection (Figure 1).

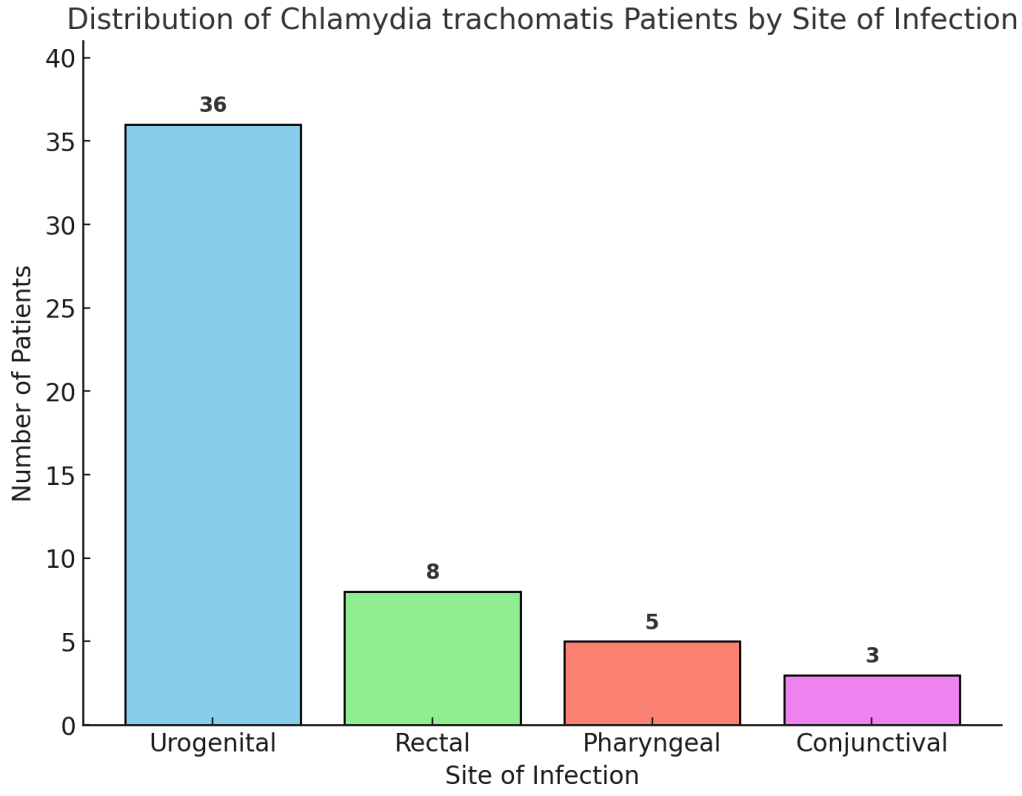

Figure 1. Distribution of patients according to the site of Chlamydia trachomatis infection

A statistically significant increase in serum IL-6 concentrations was observed in patients with Chlamydia trachomatis infection (18.6 ± 5.2 pg/ml) when compared with the control group (12.4 ± 4.1 pg/ml, P < 0.03), as shown in table 2. This outcome indicates that the cytokine is markedly induced during infection, underscoring its involvement in the host inflammatory response. The elevation of IL-6, therefore, highlights its potential as a discriminative biomarker for distinguishing affected individuals from healthy counterparts, thereby reinforcing its possible clinical application in diagnostic practice.

Table 2. Evaluation of IL-6 levels between patients with Chlamydia trachomatis and control participants

Groups	No.	IL-6 (pg/ml) Mean ± SD	T Test (P Value)
Patient	52	18.6 ± 5.2	P < 0.03 (S)
Control	48	12.4 ± 4.1	

S: Significant at P<0.001

Analysis of serum IL-6 levels according to the site of Chlamydia trachomatis infection revealed significant differences among patient subgroups (P = 0.02). The highest IL-6 levels were observed in urogenital infections (19.2 ± 5.1 pg/ml), followed by rectal infections (17.5 ± 4.6 pg/ml). In contrast, pharyngeal (14.8 ± 3.9 pg/ml) and conjunctival infections (13.6 ± 3.2 pg/ml) showed comparatively lower levels (table 3).

Table 3. Comparison in IL-6 levels in patients' groups based on site of Chlamydial infection

Age Sub-groups	Freq.	IL-6 (pg/ml) Mean ± S.D	F test	T test P-value
Urogenital	36	19.2 ± 5.1 (A)	3.72	0.02 (S)
Rectal	8	17.5 ± 4.6 (B)		
Pharyngeal	5	14.8 ± 3.9 (C)		
Conjunctival	3	13.6 ± 3.2 (C)		

A, B, C express significant difference at p <0.05; S: Significant at P<0.05

Table 4 reveals the area under the curve (AUC) is about 0.84 (p < 0.001). This was measured when the cutoff value was determined above 15.5 pg/ml, IL-6 resulted in a sensitivity of 80.8% and a specificity of 77.1%, thereby confirming its reliability as a diagnostic marker for identifying positive cases. Such diagnostic performance supports the inclusion of IL-6 in laboratory-based screening strategies for C. trachomatis infection, particularly in settings where rapid and cost-effective diagnostic tools are needed (figure 2).

Table 4. Receiver operating characteristic (ROC) analysis of IL-6 for the diagnosis of Chlamydia trachomatis infection

Biomarker	(AUC)	p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
IL-6	0.84	0.84	0.84	0.84	0.84

AUC: Area Under the curve

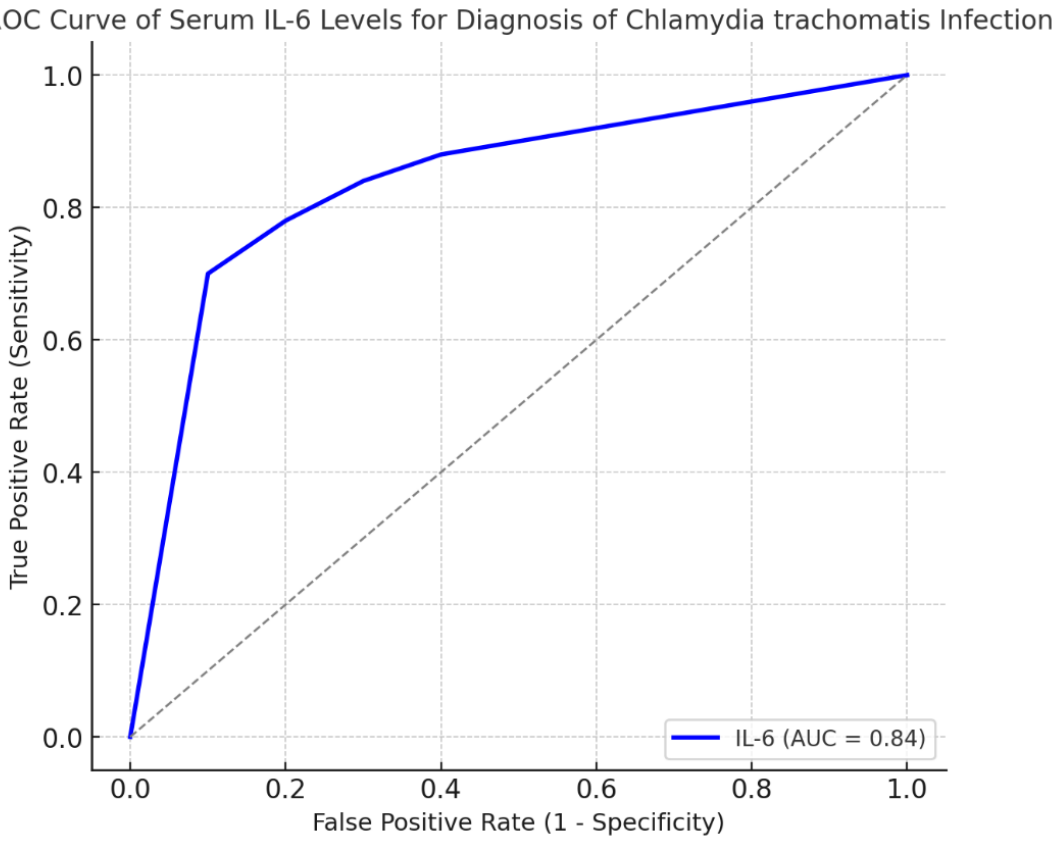

Figure 2. ROC Curve of Serum IL-6 Levels for diagnosis of Chlamydia trachomatis infection

Discussion

The present study has revealed that patients infected with Chlamydia trachomatis exhibited significantly higher serum IL-6 concentrations, averaging 18.5 ± 4.2 pg/ml, whereas healthy controls demonstrated lower levels, with a mean of 11.2 ± 3.6 pg/ml. The discriminative capacity of IL-6 was further confirmed through receiver operating characteristic (ROC) curve analysis, which resulted in an area under the curve (AUC) value of 0.84. When a cutoff value of 15.5 pg/ml was applied, the sensitivity and specificity were calculated as 82.7% and 79.1%, respectively, indicating that IL-6 possesses strong potential for differentiating between infected and non-infected individuals.

Interleukin 6 (IL-6) is synthesized within minutes and hours after infections and tissue injuries, contributing, in concert with other cytokines, to human defense through immune response activation, augmentation of hematopoiesis, and acute phase reactants (Tanaka et al., 2016; Alfahham et al., 2024). The elevated concentrations observed in C. trachomatis patients in this investigation are consistent with the pathogen's capacity to stimulate IL-6 production through toll-like receptor (TLR) and NF-κB signaling pathways, mechanisms well-established in prior studies (O'Connell & Balaji, 2020).

Chlamydial infection similarly triggers the production of proinflammatory cytokines such as IL-6 and IL-8, leading to lysis of epithelial cells and IL-1β release, in addition to prolonged local inflammation and recruitment of T-cell. While the cellular and molecular pathways linking STI-associated inflammation with increase IL-6, there are scare and variant data on the biological impacts of chlamydia /gonorrhea infection on the pathways linking mucosal inflammation with sexually transmitted infections in human (Gitsels et al., 2019). Elevated genital IL-6 has been previously associated with reproductive complications, including pelvic inflammatory disease and tubal infertility (Morrison et al., 2020).

ROC curve results reinforce the potential of IL-6 as a clinically useful biomarker. Similar trends have been documented in other infectious contexts: IL-6 levels have been linked to disease severity in viral pneumonia (Chen et al., 2020), systemic inflammation in sepsis (Balcioglu et al., 2018), and elevations in sexually transmitted infections such as gonorrhea and Mycoplasma genitalium (Liu et al., 2019).

Previously published studies have suggested that IL-6 may be associated with the persistence and chronic

nature of infections. A positive correlation between sustained IL-6 concentrations and prolonged bacterial presence has been documented by Budai et al. (2019), indicating that this cytokine could serve as a marker for monitoring disease progression. This association is particularly pertinent to *Chlamydia trachomatis*, which frequently remains asymptomatic yet poses long-term risks, such as infertility and ectopic pregnancy (Vodstrcil et al., 2017). Further evidence supporting the involvement of IL-6 in the immune response to *C. trachomatis* infection was provided by Geisler et al. (2017), who observed that cervical epithelial cells released substantial amounts of IL-6 upon pathogen exposure, thereby activating downstream inflammatory signaling pathways. Additionally, Masson et al. (2016) demonstrated that elevated IL-6 levels in genital secretions were linked to increased susceptibility to HIV infection, highlighting its dual function as both an immune mediator and a factor contributing to comorbid risk. The findings of the present study corroborate these earlier reports by quantifying systemic IL-6 levels and validating its potential diagnostic significance.

Despite the strengths of this study, many limitations merit attention. The sample size, although informative, was modest, and future multicenter studies are required to validate the diagnostic threshold of 15.5 pg/ml. Another limitation lies in IL-6's lack of specificity; it is a sensitive inflammatory marker but elevated levels are also characteristic of diverse infections and systemic conditions (Tanaka et al., 2016). Thus, the incorporation of IL-6 into a diagnostic panel—possibly alongside markers such as IL-8 or C-reactive protein (CRP)—may enhance overall accuracy. Finally, longitudinal investigations are necessary to explore whether IL-6 levels decline with treatment or correlate with long-term outcomes, thereby clarifying its role in patient management (Li et al., 2021).

In summary, this study revealed that serum IL-6 is significantly elevated in patients with *C. trachomatis* infection, with strong diagnostic performance reflected by an AUC of 0.84. These results contribute to the growing evidence that IL-6 is not only a marker of acute immune response but also a potential predictor of infection-related complications. Broader validation may establish IL-6 as a valuable tool for early detection, risk stratification, and monitoring in *C. trachomatis* management.

Conclusion:

In summary, this investigation revealed a marked rise in serum IL-6 concentrations among individuals diagnosed with *Chlamydia trachomatis* infection compared to healthy controls. The analysis indicated that IL-6 possesses substantial discriminative capacity for diagnostic purposes, with particularly strong performance in urogenital infections. These findings correspond with earlier evidence that has consistently identified IL-6 as a key biomarker associated with bacterial pathogenesis. Rather than relying on IL-6 alone, future directions should aim to incorporate it into broader diagnostic frameworks in conjunction with molecular assays. Such an approach would likely improve the accuracy of early detection and facilitate more effective clinical management of *C. trachomatis* cases.

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