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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 is one of the most common sexually transmitted bacterial pathogens and often asymptomatic but can cause negative consequences, including infertility. Identifying robust biomarkers has been highlighted as necessary for improving early detection strategies. **Aim**: The aim of this study was to assess the possible usefulness of circulating interleukin-6 (IL-6) levels in the diagnosis of Chlamydia trachomatis infection. **Methods**: A case–control study with 52 cases of laboratory-confirmed C. trachomatis infection and 48 apparently healthy controls was conducted. We measured serum IL-6 levels by ELISA. Independent samples t-tests were performed for between-group differences and analysis of variance (ANOVA) was used to analyze site differences regarding the factors associated with improving quality of life. Furthermore, the ability of IL-6 to differentiate between infected and non-infected subjects was assessed by receiver operating characteristic (ROC) curve analysis. **Results**: The IL-6 levels were significantly higher in patients (18.6 ± 5.2 pg/ml) than those in controls (12.4 ± 4.1 pg/ml, $p < 0.03$). We also determined serum IL-6 levels by anatomical infection sites showing that they were the highest in urogenital infections (19.2 ± 5.1 pg/ml) and moderately lower for rectal infections (17.5 ± 4.6 pg/ml). The area under the curve (AUC) was 0.84, with an ideal diagnostic threshold set at 15.5 pg/ml, with a sensitivity of 80.8% and specificity of77.1%. **Conclusions**: These results indicate that the levels of IL-6 is significantly higher in those infected with Chlamydia trachomatis, which suggests as a potential monitoring biomarker at sites of urogenital infection.

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

Introduction

Sexually transmitted diseases (STDs) represent a major global health challenge with significant social, economic and public health consequences. The infections have invoked his humanit- of many decades, going above and beyond the borders (both geographically and socially) with Chlamydia trachomatis the most prevalent 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. More importantly, IL-6–deficient mice have a higher susceptibility to systemic *Escherichia coli* infection (Thirone et al. 1996), as these mice exhibited significantly increased bacterial loads and mortality when compared with controls suggesting an important protective role of IL-6 in host defense. Furthermore, rapid induction of IL-6 at mucosal surfaces and in serum regulates these levels that correlate with severity of infection (de Man et al., 1989) murine models of gram-negative bacterial challenge.

Focusing on Chlamydia trachomatis in particular, some recent studies are now giving insight into the specific role of IL-6 in local immunity. In vitro cell culture studies conducting on epithelial cells or both epithelial and immune cells infected with C. trachomatis resulted in elevating IL-6 secretion. As another example, it was found that in vitro infection of HeLa epithelial cells and monocyte-like THP-1 cells resulted in sustained IL-6 (and IL-8) secretion for 72 h or more post-infection especially during co-culture where this was identified as a continuous form of inflammatory signaling even when infection is under partial control (persistent persistence) such as occurs during results from some antibiotics, Mpiga et al. dd). Contrastingly, the production of IL-6 in response to live C. is variable but detectable by human primary reproductive epithelial cultures and peripheral blood mononuclear cells (PBMCs) from donors [15-20].

tra- chromatis, or chlamydial stress-response antigens (HtrA, Tsp) (Russell et al., 2014).

Despite these insights, there remains a lack of literature with regard to the ability of serum IL-6 levels (i.e.systemically circulating IL-6) to distinguish between cases and controls for individuals with C. trachomatis infection. Logically, ie: COI of serum IL-6, sensitivity and specificity? At what point in the course of infection does it become detectable? What is the baseline in uninfected populations, and is there concordance within individuals? Do host factors (age, co-infections, immune status) that induce IL-6 independent of infection?

If diagnostic discriminative power is adequate, IL-6 may provide important ancillary utility: efficient screening, triage in clinical environments, or point-of-care-test (POCT) testing in lower resource settings. The purpose of this study is to determine the diagnostic value of IL-6 level in serum for a Chlamydia trachomatis infection. Here we aimed (a) to compare the concentrations of serum IL-6 between infected and uninfected subjects,

(b) evaluate the performance of serum IL-6 to discriminate infection status through sensitivity, specificity and receiver operating characteristic (ROC) curve analysis; and (c) determine whether serum IL-6 could be a practical biomarker in routine care or screening program. The findings could help clarify the degree to which IL-6 can be used as an adjunct to current diagnostic modalities, particularly in settings with less availability of molecular diagnostics.

Patients and Methods

Study Design and Setting

The study was a case–control study that took place between August 2024 and February 2025 at Hussein Teaching Hospital in Karbala, Iraq. The hospital is a tertiary referral center for Karbala and adjacent provinces that only recently gained access to information regarding the entire 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 contrast, 8 (15.4%) were from rectal infections, 5 (9.6%) were pharyngeal infections and only 3 (5.8%) had conjunctival involvement. This distribution is consistent with the established epidemiology of C. trachomatis, in which urogenital transmission predominates.

Extragenital sites such as the rectum and oropharynx have relatively lower frequencies, possibly due to differences in risk of exposure, sexual practices, and undertesting since these infections frequently go undiagnosed. These results support the need for clinical prioritization of urogenital screening before also considering extragenital testing in order to limit undetected reservoirs of persistent infection, in which urogenital transmission predominates (Figure 1).

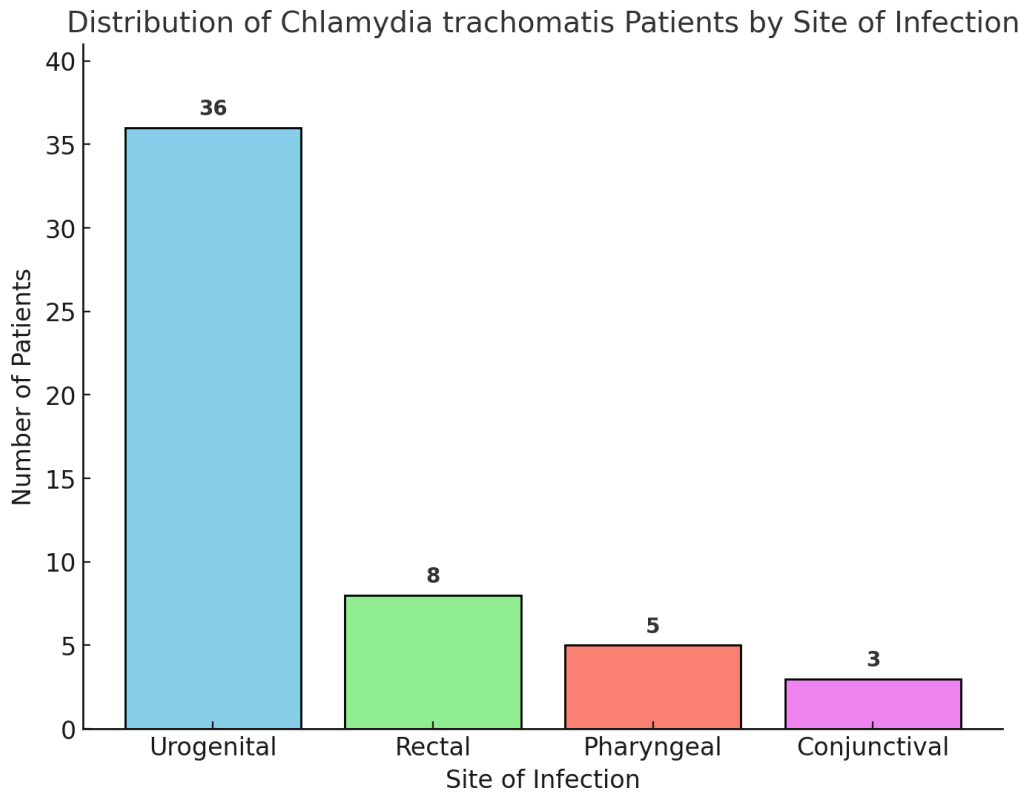

Figure 1. Distribution of patients according to the site of Chlamydia trachomatis infection

Patients with Chlamydia trachomatis infection had significant higher serum IL-6 concentrations (18.6 ± 5.2 pg/ml) than the controls (12.4 ± 4.1 pg/ml, P < 0.03), as illustrated in table 2 The fact that the cytokine is strongly induced in infection highlighting its participation in the immune response of the host. Elevated levels of IL-6, therefore, define its potential as a useful biomarker for discriminating between patients with the disease and normal individuals furthering its clinical relevance in diagnosis.

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

Serum levels of IL-6 were found to be significantly different between the patient subgroups according to the site of Chlamydia trachomatis infection (P = 0.02). Among the infections studied, urogenital infections had (19.2 ± 5.1 pg/ml), followed by rectal infections (17.5 ± the highest levels of IL-6.

4.6pg/ml). Pharyngeal(14.8±3.9pg/ml)and conjunctival infections (13.6 ± 3.2 pg/ml)had lower levels than genital tract(P<0.012) (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). When the cutoff value was established at more than 15.5 pg/ml, IL-6 yielded a sensitivity and specificity of 80.8% and 77.1%, respectively to confirm its reliability as an accessary diagnostic marker for identifying positive cases. These diagnostic performances suggest the potential utility of IL-6 for laboratory-based screening strategy of C. trachomatis infection, particularly in settings requiring a prompt and cost-effective diagnostic device (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

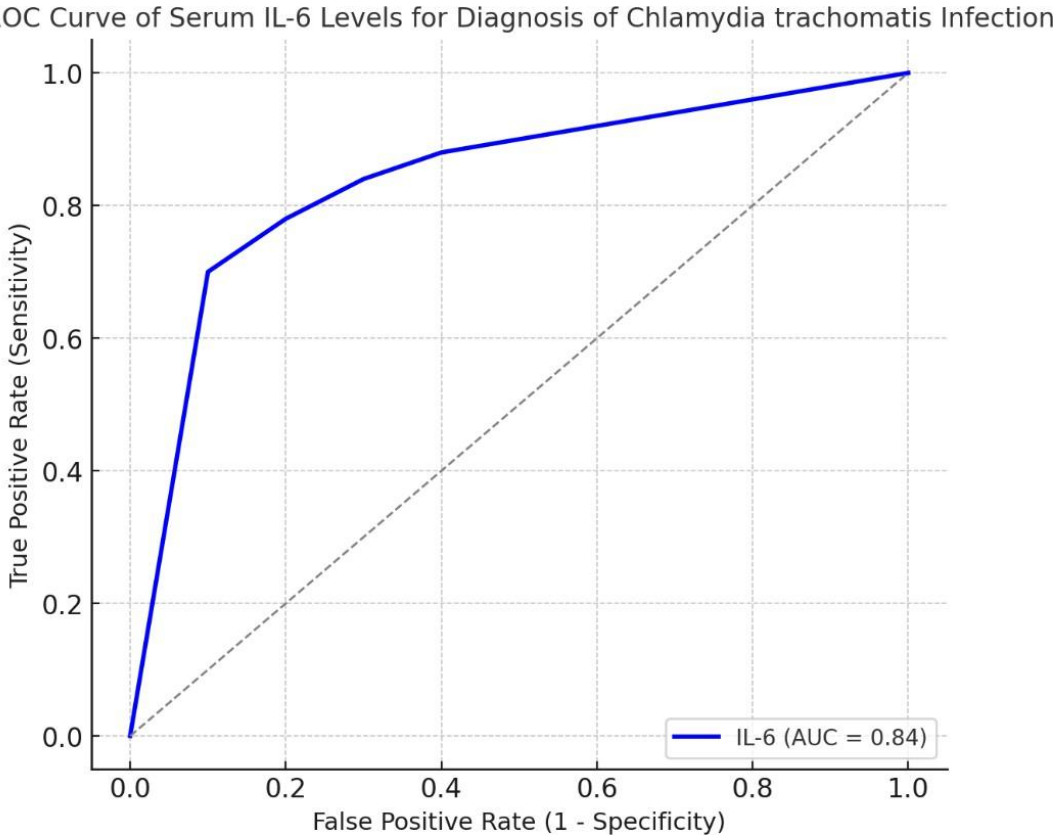

Figure 2. ROC Curve of Serum IL-6 Levels for diagnosis of Chlamydia trachomatis infection

Discussion

In the present study, patients with Chlamydia trachomatis infection had a much higher serum IL-6 level than healthy controls, 18.5 ± 4.2 pg/ml and 11.2 ± 3.6 pg/ml respectively. Receiver operating characteristic (ROC) curve analysis was performed assessing the discriminative potency of IL-6, which yielded an AUC value of 0.84. IL-6 shows the great potential for differentiating between infected and non-infected individuals when a cutoff value of 15.5 pg/ml was used, yielding a sensitivity of 82.7% and specificity of 79.1%.

IL-6 is synthesized minutes to hours after infections and tissue injuries, together expert induced by pathogen associated molecular pattern (PAMPs) or damage-associated molecular pattern (DAMPs), being recognized as pro-inflammatory cytokine through the activation of immune system response promoting myeloblastogenesis and further proof that inactivated disease imposes whole body signals promoting 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). Increased genital IL-6 has been previously linked with reproduction problems such as pelvic inflammatory disease and tubal infertility (Morrison et al., 2020).

ROCcurveresultsreinforcethepotentialofIL-6asa clinically useful biomarker. Analogous trends have been reported in other infectious context: in viral pneumonia, IL-6levels have been correlated with disease severity (Chen etal., 2020), systemic inflammation in sepsis (Balcioglu etal., 2018), and elevations in gonorrhea and Mycoplasma genitalium sexually transmitted infections (Liu et al., 2019).

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

nature of infections. Budai et al. documented a correlation between sustained IL-6 concentrations and persistent bacterial viability. This may suggest that this cytokine could potentially be a marker of disease progression to monitor (2019). This association is especially relevant for Chlamydia trachomatis, which often goes unnoticed but can induce abdominal long-term complications such as infertility and ectopic pregnancy [61]. Geisler et al. further corroborated the role of IL-6 in the immune response to C. trachomatis infection (2017) noted that large amounts of IL-6 were secreted from cervix epithelial cells when exposed to pathogens, which then turned on inflammatory signalling cascades downstream. Additionally, Masson et al. Another correlation study (2016) showed that elevated levels of IL-6 in genital secretions correlated with increased HIV susceptibility underscoring the contribution of this cytokine as an immune mediator and a comorbid risk factor. This study confirms these previous observations in a more quantitative manner by measuring IL-6 in the systemic circulation and thus supports its potential as a diagnostic biomarker among non-fatal acute myocardial infarction patients.

The limitations of this study are numerous despite the strengths. While informative, the sample size was small and future multicenter studies are necessary to corroborate on the 15.5 pg/ml diagnostic threshold. Another limitation concerns the low specificity of IL-6: even though it is a sensitive inflammatory marker, high levels are also present in various infections and systemic disorders (Tanaka et al., 2016). Therefore, the addition of IL-6 into a diagnostic panel—potentially combining it with other inflammatory markers like IL-8 or C-reactive protein (CRP)—could improve overall specificity [12]. Third, more longitudinal studies are needed to uncover whether IL-6 decreases with treatment or relates to long-term outcomes helping guiding its place in the management of patients (Li et al., 2021). ConclusionIn conclusion, this study found markedly higher levels of serum IL-6 levels in C. trachomatis infection patients with good diagnostic performance (area under the curve [AUC] 0.84). These findings add to the increasing evidence supporting the notion that IL-6 is a not just a marker of acute immune response but a disease predicting factor in infection-induced complication.

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Conclusion:

This study showed that serum IL-6 levels were significantly higher in patients with Chlamydia trachomatis infection than in healthy controls. Former studies could display good discriminative energy for diagnostic values of IL-6, specifically with the robust performance particularly observed within the diagnosis of cases having urogenital infections. These observations further corroborate prior evidence that IL-6 represents a central pathophysiological signature of the bacterial disease states. Moving forward, IL-6 should not be interpreted in isolation but should instead be integrated with array or high-throughput molecular assays. This approach would potentially lead to higher sensitivity of early detection and will allow for a better clinical management of C. trachomatis cases.

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