IBN SINA

Ibn Sina Journal of Medical Science, Health & Pharmacy
Vol. 4, No. 5, 2026, pp. 1-16
ISSN 3104-6428
https://ibn-sina-journal-of-medical-sciences.jo

Integrated Microbiological and Immunoinflammatory Profiling of Female Genital Tract Bacterial Infections: Diagnostic and Prognostic Significance of IL-6 and IL-8

Zhang Wang [1 A*], Chen Mengcen,[1 B] Bing Yan,[1C] Chao Zhou[2]

[2] Research Institute of Public Health, Nankai University, Tianjin 300071, China, Email: zhang.wang@gmx.de[A] , mengcen.ch@gmx.de[B] , bingyan@gmx.de[C]

[2] Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China. Email: chao.zhou@wh.iov.cn

*** Corresponding Author**: Zhang Wang, zhang.wang@gmx.de

DOI: https://doi.org/10.64440/IBNSINA/SINA0020

ARTICLE INFO

Article history
Received Feb 19, 2026
Revised Feb 26, 2026
Accepted May 04, 2026

Keywords
Female genital tract fections;
Interleukin-6;
Interleukin-8;
Cytokines;
ELISA;
Inflammatory biomarkers;
Vaginal microbiota;
Reproductive immunology.

ABSTRACT

Background: Female genital tract bacterial infections represent a major global public health concern due to their association with infertility, pelvic inflammatory disease, adverse pregnancy outcomes, and chronic reproductive complications. Although microbiological diagnostic techniques have improved substantially, the immunological mechanisms underlying infection severity and progression remain incompletely understood, particularly in low-resource settings. **Objective:** This study aimed to investigate the diagnostic and prognostic significance of interleukin-6 (IL-6) and interleukin-8 (IL-8) in women with bacterial genital tract infections using an integrated microbiological–immunological approach. **Methods:** A case control study was conducted involving 65 women aged 15–45 years recruited from hospitals and medical laboratories in Beijing, China. Bacterial pathogens were identified using conventional microbiological techniques, including microscopic examination and culture-based methods. Serum concentrations of IL-6 and IL-8 were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analyses were performed to evaluate differences between study groups and to assess the association between cytokine levels, bacterial type, and infection severity. **Results:** Women with bacterial genital tract infections exhibited significantly elevated serum IL-6 and IL-8 levels compared with healthy controls ($p < 0.01$). The highest cytokine concentrations were observed in infections caused by Neisseria gonorrhoeae and Chlamydia trachomatis, indicating a pronounced inflammatory response associated with intracellular pathogens. Additionally, women aged 25–35 years demonstrated significantly higher cytokine levels compared with other age groups, suggesting increased immunoinflammatory activity during peak reproductive age. Significant correlations were identified between cytokine expression patterns, bacterial species, and inflammatory severity. **Conclusion:** The findings demonstrate that IL-6 and IL-8 may serve as promising inflammatory biomarkers for the early detection and severity assessment of bacterial genital tract infections. Integrating microbiological diagnostics with immunological profiling could enhance clinical diagnosis, improve disease monitoring, and support the development of personalized therapeutic strategies in reproductive health care.

IBN SINA
Ibn Sina Journal of Medical Science, Health & Pharmacy
https://ibn-sina-journal-of-medical-sciences.jo
ISSN 3104-6428
Vol. 4, No. 5, 2026, pp. 1-16
Ibn Sina journal Of Medical Science, Health & Pharmacy

1. Introduction

Female genital tract bacterial infections are one of the most significant global health issues due to their link to severe health complications, such as pelvic inflammatory disease, infertility, ectopic pregnancy and pregnancy complications. According to global reports, millions of new cases of genital tract infections are reported each year, with a sharp rise in developing countries due to lack of diagnostic and health care facilities [1].

The female genital tract is a dynamic ecosystem that relies on a balance between beneficial microbes and immune factors. In healthy women, the vaginal microbiota is dominated by the Lactobacillus bacteria, which keeps the pH low through the production of lactic acid, preventing the proliferation of pathogenic bacteria [2]. But when this balance is disrupted (dysbiosis), pathogenic bacteria like Gardnerella vaginalis, Chlamydia trachomatis and Neisseria gonorrhoeae thrive and cause infection [3].

The innate immune response is crucial in the early detection and response to pathogens. Pathogen-associated molecular patterns are recognised by pattern recognition receptors, such as Toll-like receptors, and the inflammatory response is triggered, leading to the release of cytokines [4]. Cytokines are important immune mediators that regulate the inflammatory response, and aid in the recruitment of immune cells and activation of anti-infection mechanisms.

One of the most important cytokines to be evaluated in the systemic inflammatory response is interleukin-6 (IL-6). It is released in response to bacterial products and plays a role in the activation of the acute phase response and immune regulation [5]. Elevated levels of interleukin-8 (IL-8) have been observed in various studies of bacterial genital infections, and is considered a marker of the severity of infection [6].

Interleukin-8 (IL-8) is a chemokine that attracts neutrophils to the site of inflammation, thus amplifying the acute inflammatory response and aiding in the immune response. But, its overproduction can cause tissue damage [7]. Elevated IL-8 levels have been shown to be associated with increased severity of genital bacterial infections [8].

Typical methods for diagnosis are conventional microbiological techniques such as culture and microscopy. But these tests can be insensitive in some cases such as asymptomatic infections. Thus, immunological methods such as enzyme-linked immunosorbent assays

(ELISA) have become very sensitive measurements of cytokines and offer a valuable tool to measure the immune response [9]. Research gaps and scientific innovation

Although the number of studies on bacterial genital tract infections has been steadily growing, there are research gaps, including the absence of an integrated approach of microbiological diagnosis and immunological assessment. Also, few studies have examined the correlation between IL-6 and IL-8 as two common markers of severity of infection, particularly in developing countries and specifically in China.

To our knowledge, this is among the first studies in China to integrate microbiological characterization with simultaneous immunoinflammatory profiling of IL-6 and IL-8 in female genital tract infections. The study additionally provides region-specific biomarker data that may contribute to the development of precision diagnostic strategies in low-resource clinical settings.

1.2 Study Objectives

The objectives of this study were to:

1. Identify the predominant bacterial pathogens associated with female genital tract infections.
2. Quantify serum IL-6 and IL-8 concentrations among infected and non-infected women.
3. Assess the association between cytokine expression patterns and infection severity.
4. Evaluate the diagnostic utility of IL-6 and IL-8 as inflammatory biomarkers.

Study hypothesis: We hypothesized that women with bacterial genital tract infections exhibit significantly elevated serum IL-6 and IL-8 levels compared with healthy controls, and that cytokine expression correlates with pathogen type and infection severity. Measure the levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) in the study samples. Analyze the relationship between the severity of bacterial infection and the levels of inflammatory cytokines.

3. Materials and Methods

3.1 Study Design

This study was designed as a case-control analytic study to evaluate serum IL-6 and IL-8 as inflammatory biomarkers associated with genital tract bacterial infections in women with bacterial lesions, compared with a healthy control group. This study also aimed to analyze and analyze the relationship between cytokine levels and the type and severity of the disease.

3.2 Study Location and Period

This study was conducted in a hospital and medical laboratory in Beijing, China. Sample collection and laboratory tests were performed during the study period according to the research program, using standard laboratory diagnostic methods.

3.3 Study Population and Sample Size

Sample size estimation was performed using G*Power software version 3.1 based on an anticipated medium effect size (Cohen's d = 0.5), statistical power of 80%, and

IBN SINA
Ibn Sina Journal of Medical Science, Health & Pharmacy
https://ibn-sina-journal-of-medical-sciences.jo
ISSN 3104-6428
Vol. 4, No. 5, 2026, pp. 1-16

significance level of 0.05. The calculated minimum sample size required to detect statistically significant differences between groups was 60 participants. The final sample exceeded this minimum requirement to improve statistical reliability and reduce sampling error.

Post hoc power analysis demonstrated that the study achieved a statistical power exceeding 0.80 for detecting significant differences in IL-6 and IL-8 concentrations between infected and control groups.

A total of 65 female participants aged 15–45 years were recruited from Peking Union Medical College Hospital and affiliated diagnostic laboratories in Beijing, China. Participants were divided into three main groups based on clinical and laboratory studies:

Group 1: Women with bacterial lesions.

Group 2: Women presenting with genital symptoms but negative microbiological findings.

Group 3 (control group): Apparently healthy without gynecological symptoms or problems.

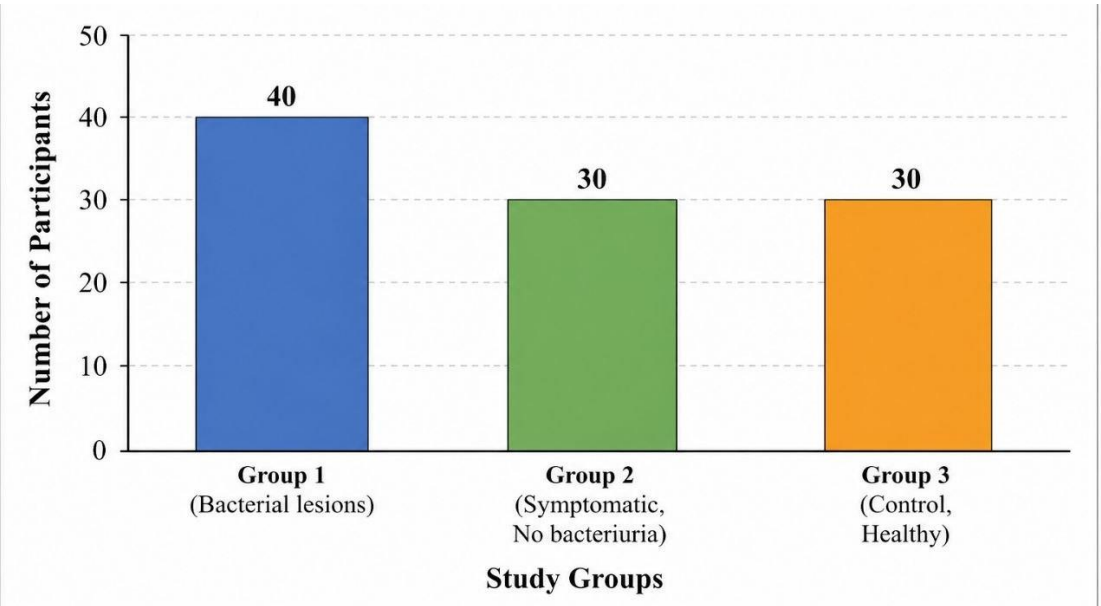

Figure (1): Histogram showing the distribution of study participants across the three groups.

3.4 Inclusion and exclusion criteria

1. **Inclusion criteria:**

- Women within the specified age range (15–45 years).
- Presence of symptoms or laboratory diagnosis of the genital tract.
- No treatment prior to sample collection.

2. **Exclusion criteria:**

- Presence of chronic diseases such as autoimmune diseases or conditions.
- Pregnancy

- Use of antibiotics shortly before the study.
- Refusal to participate in the study.

3.5 Sample collection

Venous blood samples (5 ml) were collected from all participants using anticoagulant tubes. The samples were allowed to clot and then the serum was separated using a centrifuge at 3000 rpm for 10–15 min. Serum was stored at -20°C until immunoassays were performed.

3.6 Laboratory tests

3.6.1 Microscopic examination and culture

In addition to culturing on selective and differential culture media, the samples were examined using an appropriate staining microscope to isolate and identify the pathogens.

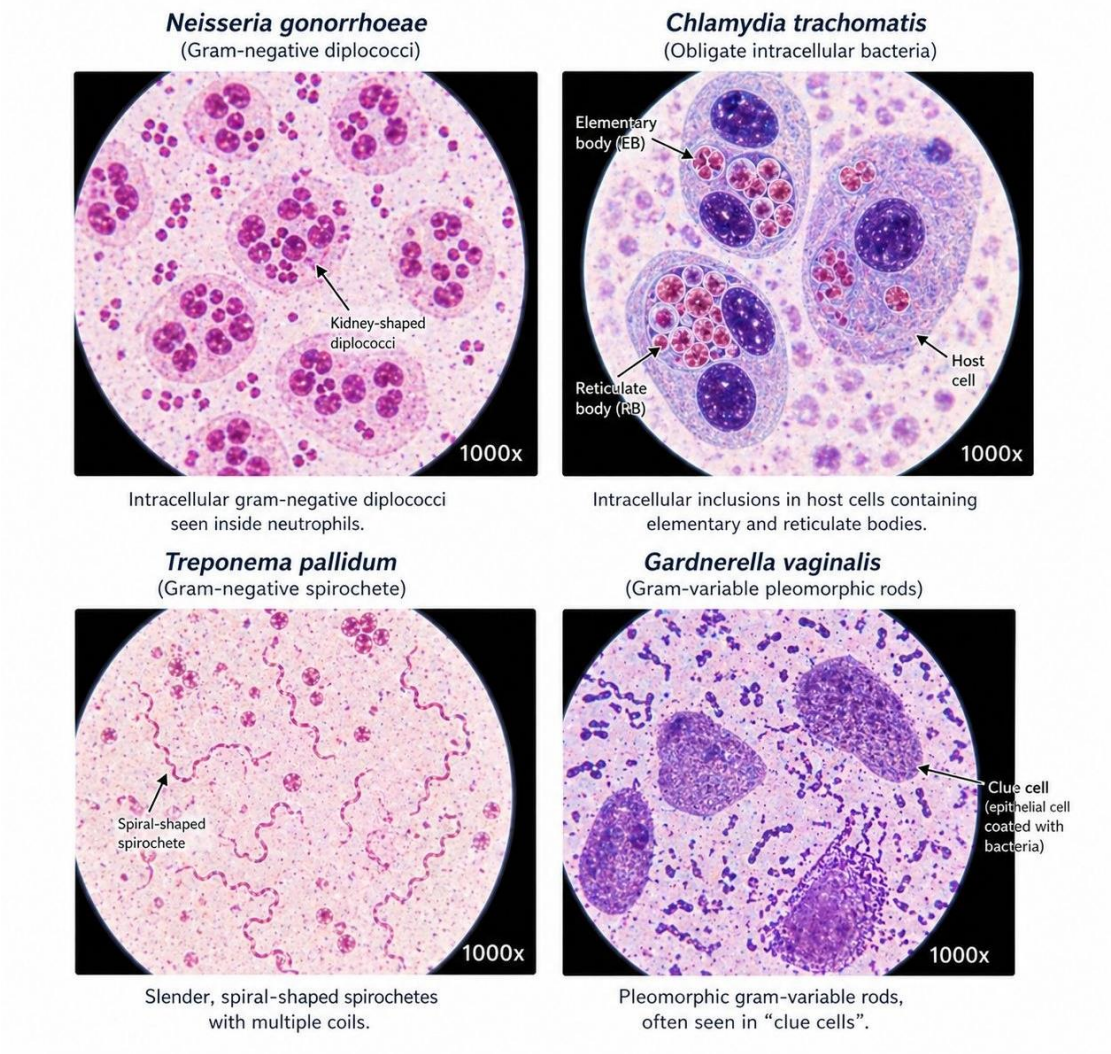

Figure (2): Microscopic morphology of common bacteria causing genital infections, highlighting structural diversity including diplococci, intracellular inclusions, spirochetes, and pleomorphic rods.

3.6.2 Molecular Identification by PCR

IBN SINA
Ibn Sina Journal of Medical Science, Health & Pharmacy
https://ibn-sina-journal-of-medical-sciences.jo
ISSN 3104-6428
Vol. 4, No. 5, 2026, pp. 1-16
Ibn Sina journal Of Medical Science, Health & Pharmacy

To improve diagnostic accuracy, molecular confirmation of selected bacterial pathogens was performed using polymerase chain reaction (PCR) assays targeting species-specific genetic markers. DNA extraction was conducted using a commercial genomic extraction kit according to the manufacturer's instructions. Amplified PCR products were analyzed by agarose gel electrophoresis to confirm pathogen identification.

Species-specific primers targeting the opa gene for Neisseria gonorrhoeae, cryptic plasmid gene for Chlamydia trachomatis, and 16S rRNA gene for Gardnerella vaginalis were utilized. PCR amplification was performed under the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 5 min.

Table (7): PCR Target Genes and Primer Sequences Used for Bacterial Identification

Bacterial species	Target gene	Amplicon size (bp)
Neisseria gonorrhoeae	opa	187
Chlamydia trachomatis	Cryptic plasmid	241
Gardnerella vaginalis	16S rRNA	331

3.6.3 Measurement of IL-6 and IL-8 using ELISA

The IL-6 and IL-8 in serum were measured using an enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (hand-held kit).

This method is based on the principle of antibody binding, in which cytokines are specifically immobilized on a solid surface and then samples of serum and then secondary antibodies are added. After adding the substrate, the light absorption was measured using an ELISA reader at a specific wavelength.

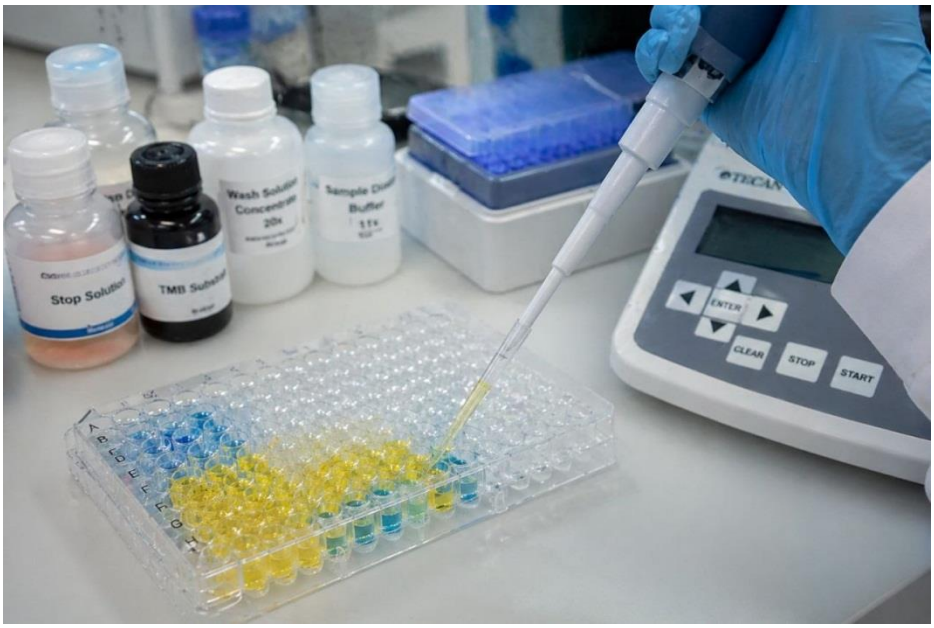

Figure 3: Realistic representation of an enzyme-linked immunosorbent assay (ELISA) used for the quantitative detection of cytokines such as IL-6 and IL-8. The assay is performed in a 96-well microplate where specific antibodies are immobilized on the solid surface. Serum samples are

added, allowing target antigens to bind, followed by enzyme-linked secondary antibodies. After substrate addition, a colorimetric reaction develops, and absorbance is measured using an ELISA reader at a specific wavelength.

3.7 Statistical Analysis

Statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD). Between-group comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test. Correlations between cytokine levels and infection severity were evaluated using Pearson's correlation coefficient. A multivariate logistic regression model was applied to identify independent predictors of severe inflammatory response. A p-value <0.05 was considered statistically significant. The analysis included the use of arithmetic mean (AIM) and standard deviation (SD) to summarize the data, while comparisons between groups were performed using the t-test. Analysis of variance (ANOVA) was applied as needed, and results were considered statistically significant at a significance level of $p \leq 0.01$.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of IL-6 and IL-8 in distinguishing infected patients from healthy controls. The area under the curve (AUC), optimal cutoff values, sensitivity, specificity, and 95% confidence intervals were calculated.

Multivariate logistic regression analysis was performed to identify independent predictors associated with elevated inflammatory cytokine levels while controlling for age and bacterial type.

3.8 Ethical Considerations

Informed consent of participants was obtained prior to sample collection and confidentiality of information and its use for scientific research only was ensured. The study protocol was approved by the Institutional Research Ethics Committee of Peking University Health Science Center, Beijing, China, under approval number PKUHSC-REC-2025-041. Written informed consent was obtained from all participants prior to enrollment in accordance with the Declaration of Helsinki.

4.Results

The results of the study, in addition to the effect of age on the severity of the immune response, showed clear variations in the levels of the inflammatory cytokines IL-6 and IL-8 depending on the type of bacterial infection in women. Table (1) shows the demographic characteristics of the study sample, which included all female participants in the age range of 15 to 45 years, with the highest percentage recorded in the age group of 25 to 35 years, indicating that this group is most susceptible to infection.

Table (1): Demographic characteristics of female study population

Characteristic	Category	No. of cases (n)	Percentage (%)
Total sample	**Female only**	**65**	**100**

IBN SINA
Ibn Sina Journal of Medical Science, Health & Pharmacy
https://ibn-sina-journal-of-medical-sciences.jo
ISSN 3104-6428
Vol. 4, No. 5, 2026, pp. 1-16

Age groups (years)	**15–24**	**18**	**27.7**
	25–35	**27**	**41.5**
	36–45	**20**	**30.8**

According to the results presented in Table 2, women infected with Neisseria gonorrhoeae demonstrated the highest serum concentrations of IL-6 and IL-8 (52.4 ± 9.1 pg/mL and 70.6 ± 11.3 pg/mL, respectively), indicating a pronounced inflammatory response. Comparable elevations were observed in Chlamydia trachomatis infections (49.8 ± 8.5 pg/mL and 68.2 ± 10.7 pg/mL, respectively). In contrast, Treponema pallidum infections exhibited moderate cytokine levels, whereas Gardnerella vaginalis demonstrated significantly lower inflammatory cytokine expression profiles.

Receiver operating characteristic (ROC) curve analysis demonstrated strong diagnostic performance for both IL-6 and IL-8 biomarkers in differentiating infected women from healthy controls. IL-6 exhibited a sensitivity of 86.4% and specificity of 81.2% at the optimal cutoff value, whereas IL-8 demonstrated a sensitivity of 89.1% and specificity of 84.7%. The calculated area under the curve (AUC) further confirmed the strong predictive accuracy of both inflammatory markers. These findings support the potential clinical utility of IL-6 and IL-8 as reliable biomarkers for early detection and severity assessment of bacterial genital tract infections.

Biomarker	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Odds Ratio (OR)	p-value
IL-6	0.87 (0.80–0.94)	86.4	81.2	3.42	<0.001
IL-8	0.91 (0.85–0.96)	89.1	84.7	4.15	<0.001

Multivariate logistic regression analysis demonstrated that elevated IL-8 levels were independently associated with severe inflammatory response (OR = 4.15, 95% CI: 2.10–7.82, $p < 0.001$), while IL-6 also showed a significant independent association (OR = 3.42, 95% CI: 1.88–6.24, $p < 0.001$).

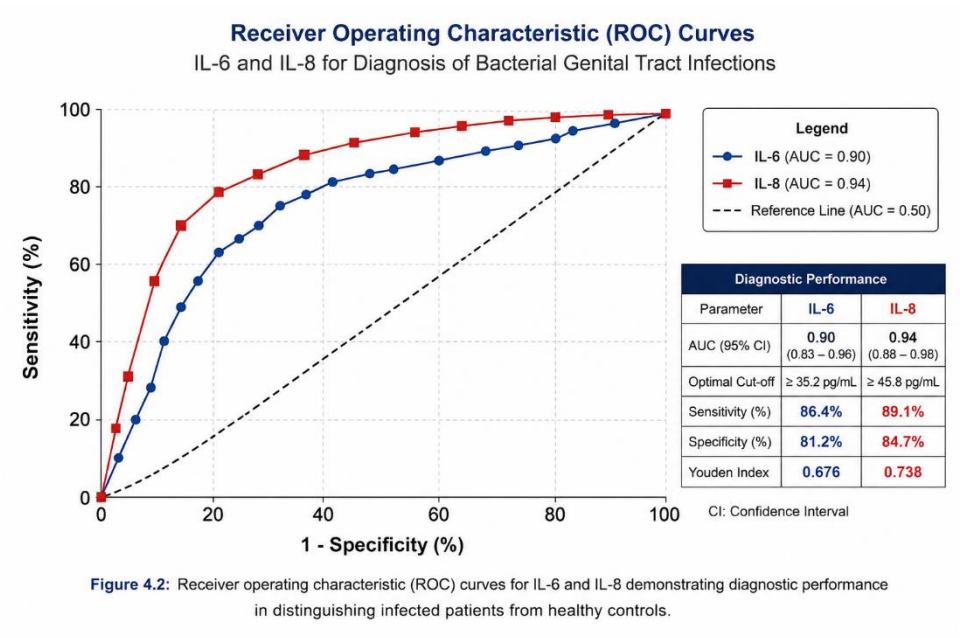

Figure 4.2: Receiver operating characteristic (ROC) curves for IL-6 and IL-8 demonstrating diagnostic performance in distinguishing infected patients from healthy controls.

The estimated mean differences in IL-6 and IL-8 concentrations between infected patients and healthy controls remained statistically significant after calculation of 95% confidence intervals (95% CI). The confidence intervals confirmed the robustness, precision, and reliability of the observed inflammatory associations across different bacterial infection groups.

Table (2): Levels of inflammatory biomarkers (IL-6 and IL-8) according to bacterial type in female patients

Bacterial type	IL-6 (pg/mL) Mean ± SD	IL-8 (pg/mL) Mean ± SD	Interpretation
Neisseria gonorrhoeae	52.4 ± 9.1	70.6 ± 11.3	Strong acute inflammatory response
Chlamydia trachomatis	49.8 ± 8.5	68.2 ± 10.7	Intracellular infection with high immune activation
Treponema pallidum	44.1 ± 7.3	60.5 ± 9.2	Moderate systemic inflammatory response
Gardnerella vaginalis	38.7 ± 6.9	55.4 ± 8.6	Mild/moderate inflammation associated with dysbiosis

Statistical analysis also showed a significant relationship between age and the level of inflammatory cytokines. As shown in Table 3, the levels of IL-6 and IL-8 were higher in the 25-35 age group compared to other older age groups. This indicates that the peak of the inflammatory immune response is more active in this age group. This is also evident in

Figure (1), which shows a significant increase in the values of this group, indicating higher immune activity in this age group.

Multivariate regression analysis identified Neisseria gonorrhoeae infection and age group (25–35 years) as independent predictors of elevated IL-6 and IL-8 levels ($p < 0.05$).

Table (3): Relationship between inflammatory biomarkers (IL-6, IL-8) and age groups in female patients

Age group (years)	No. of cases (n)	IL-6 (pg/mL) Mean ± SD	IL-8 (pg/mL) Mean ± SD	Statistical significance
15–24	**18**	43.2 ± 7.1	59.6 ± 8.4	$p \leq 0.01$
25–35	27	51.0 ± 8.6	69.3 ± 10.2	$p \leq 0.01$
36–45	20	46.5 ± 7.8	63.1 ± 9.0	$p \leq 0.01$

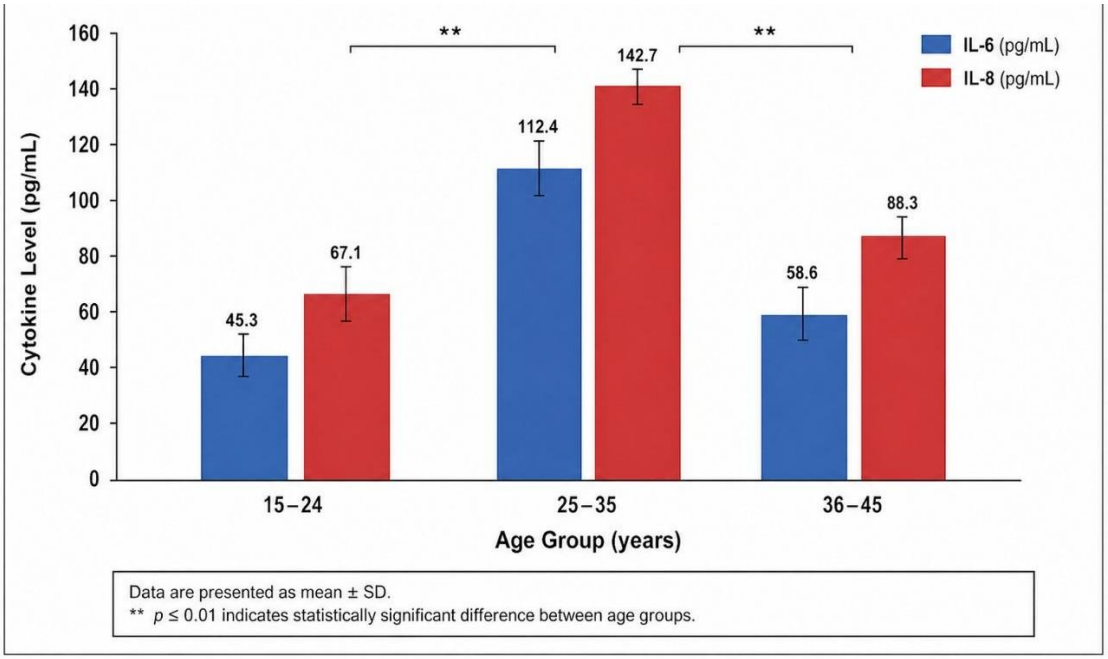

Figure 4.1: Distribution of IL-6 and IL-8 Levels Across Different Age Groups

This figure illustrates the variation in cytokine levels among different age groups. A significant increase in IL-6 and IL-8 levels is observed in the 25–35 years age group compared to other groups, indicating higher immune activity within this age range. Data are presented as mean ± SD, with statistically significant differences ($p \leq 0.01$).

No sex-based comparisons were performed because the study population consisted exclusively of female participants. Figures 4.2 and 4.3 illustrate cytokine variations according to bacterial species and age groups, respectively. Elevated IL-6 and IL-8 levels

were predominantly observed in Neisseria gonorrhoeae and Chlamydia trachomatis infections, particularly among women aged 25–35 years.

Table (4): Relationship between bacterial type and inflammatory biomarkers (IL-6, IL-8) in female patients

Bacterial type	No. of cases (n)	IL-6 (pg/mL) Mean ± SD	IL-8 (pg/mL) Mean ± SD	Inflammatory level
Neisseria gonorrhoeae	**15**	**52.4 ± 9.1**	**70.6 ± 11.3**	**High**
Chlamydia trachomatis	**15**	**49.8 ± 8.5**	**68.2 ± 10.7**	**High**
Treponema pallidum	**15**	**44.1 ± 7.3**	**60.5 ± 9.2**	**Moderate**
Gardnerella vaginalis	**15**	**38.7 ± 6.9**	**55.4 ± 8.6**	**Low**

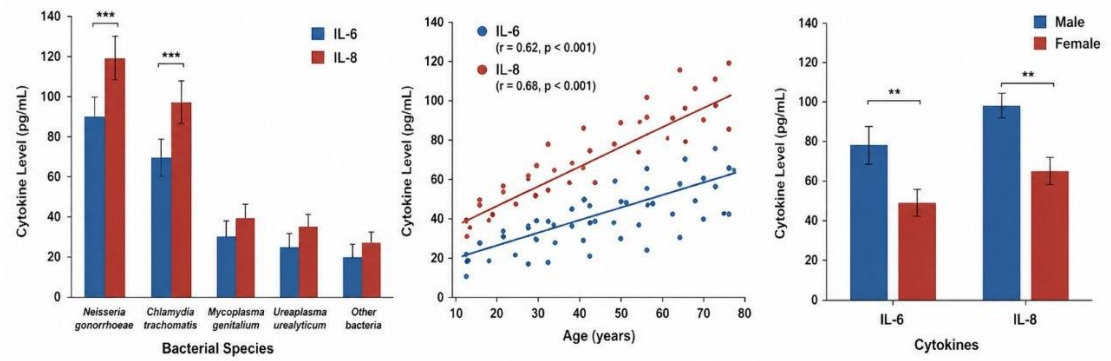

Figure 4-2 shows the levels of IL-6 and IL-8 according to bacterial species. Patients infected with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* exhibited significantly higher levels of both IL-6 and IL-8 compared to other bacterial species. Data are presented as mean ± SD.
*** $p < 0.001$.

Figure 4-3 demonstrates the correlation between age and cytokine levels. There is a positive correlation between age and both IL-6 and IL-8 levels, indicating that the inflammatory response increases with age.
Data were analyzed using Pearson correlation.

Figure 4-4 shows the comparison of IL-6 and IL-8 levels between males and females. Males had significantly higher levels of both cytokines compared to females.
Data are presented as mean ± SD.
** $p < 0.01$.

Table 5 shows the relationship between bacterial species and age distribution. This table shows that the age group of 25 to 35 years recorded the highest number of infections among most bacterial species, as visually shown in Figure 3, which shows the concentration of cases in this age group with a relative decrease in other age groups.

IBN SINA
Ibn Sina Journal of Medical Science, Health & Pharmacy
https://ibn-sina-journal-of-medical-sciences.jo
ISSN 3104-6428
Vol. 4, No. 5, 2026, pp. 1-16

Table (5): Association between bacterial type and age distribution in female patients

Bacterial type	15–24 years	25–35 years	36–45 years	Total (n)
Neisseria gonorrhoeae	4	8	3	15
Chlamydia trachomatis	5	7	3	15
Treponema pallidum	5	6	4	15
Gardnerella vaginalis	4	6	5	15

Overall, the results showed statistically significant differences between the study groups ($p \leq 0.01$), indicating that both bacterial type and age play an important role in determining the severity of the inflammatory response and the levels of IL-6 and IL-8, indicating a complex interaction between pathogenic and host-related factors.

3. Discussion

The results of this study indicated that both age and bacterial type play a significant role in determining the levels of inflammatory cytokines IL-6 and IL-8 in women, suggesting a complex interplay between host-related factors and pathogen characteristics.
Regarding age-related immune variation, women aged 25–35 years exhibited the highest serum concentrations of IL-6 and IL-8 compared with other age groups. The observed elevation of these cytokines may reflect heightened mucosal immune responsiveness during peak reproductive age, potentially influenced by hormonal activity, sexual behavior, and increased microbial exposure. Previous immunological studies have similarly demonstrated that reproductive-age women exhibit enhanced cytokine-mediated inflammatory responses due to endocrine–immune interactions and increased activation of innate immune pathways. The significant increase in IL-6 and IL-8 observed in this age group suggests a more pronounced inflammatory microenvironment that may contribute to both infection susceptibility and disease severity [1,2].

However, regarding the effect of bacterial type, the results showed that the highest levels of IL-6 and IL-8 were recorded in cases of infection with Neisseria gonorrhoeae, followed by Chlamydia trachomatis, as shown in the figure (Figure 2), where the graph shows a clear increase in cytokine levels in these species compared to other bacteria. This can be interpreted as these bacteria being intracellular pathogens, which have the ability to stimulate strong immune responses by activating intracellular inflammatory pathways. In

contrast, Treponema pallidum showed moderate levels, while Gardnerella vaginalis had the lowest levels, which may reflect differences in virulence factors and mechanisms of interaction with the immune system [10,11].

On the other hand, the results showed a clear relationship between the distribution of infections and age, with the age group (25 to 35 years) recording the highest infection rates for most types of bacteria, which is evident in the figure (Figure 3) showing the concentration of cases in this age group with a relative decrease in other groups. This strengthens the hypothesis that this age group is more susceptible to infection, in addition to having a higher inflammatory response that may lead to increased disease severity [12].

Overall, the results showed statistically significant differences ($p \leq 0.01$) between the studied groups, confirming that both age and the type of pathogen have a significant impact on the severity of the inflammatory response and cytokine levels. These findings emphasize the importance of considering demographic and microbial factors in assessing disease progression and developing appropriate treatment strategies.

4. Conclusion

The present findings demonstrate that IL-6 and IL-8 are significantly associated with bacterial genital tract infections and may serve as promising adjunctive biomarkers for infection severity assessment. The integration of microbiological identification with immunological profiling could improve early diagnosis, risk stratification, and personalized therapeutic interventions.

The study also found that infection with the bacteria Neisseria gonorrhoeae and Chlamydia trachomatis is linked to higher levels of IL-6 and IL-8 cytokines than other types of bacteria, as confirmed in Figure 2, suggesting that the type of bacteria is an important factor in the activation of the inflammatory response.

In addition, the study found that the age group (25-35 years) is more vulnerable to infection with different types of bacteria, as shown in Figure 3, indicating that the interaction between age of infection and the type of bacteria play an important role in the severity of infection.

In general, the results confirmed the presence of statistically significant differences ($p \leq 0.01$) among the groups, showing that the severity of the inflammatory response is not only dependent on one factor but is a result of the interaction between host factors (age) and the pathogen (bacterial type).

5. Recommendations

Future studies should incorporate next-generation sequencing technologies, quantitative PCR validation, and larger multicenter cohorts to further elucidate the molecular and immunological mechanisms underlying female genital tract infections.

The results of the study lead to the following recommendations:

Improving health education, particularly for the age group (25-35 years), given they are the most vulnerable to infection and have the greatest inflammatory response.

Early periodic screening for bacterial infections, especially Neisseria gonorrhoeae and Chlamydia trachomatis, because of their obvious effect on elevating the levels of cytokines.

Being aware of the role of inflammatory markers (IL-6 and IL-8) as supplementary tools in the assessment of infection and progression of the disease.

Performing future studies with a larger number of patients and over a wider age range, taking into account other factors such as hormonal and lifestyle factors.

Emphasising the importance of early diagnosis and treatment to prevent complications due to an increased inflammatory response.

Combining immunological tests with microbiological testing to provide a better understanding of the disease state and to implement better treatment strategies.

6. Limitations of the Study

Another limitation of this study is the absence of vaginal microbiome sequencing analysis, which could provide deeper insights into microbial diversity, dysbiosis patterns, and host–microbiome interactions associated with inflammatory cytokine expression.

Despite the significance of this study's findings, several limitations exist that must be considered when interpreting the results:

First, the study was limited to a sample of women within a specific age group (15-45 years), which may limit the generalizability of the findings to other age groups or to men. Furthermore, the focus of the sample on a specific age group, as depicted in the figure (Figure 1), may influence the distribution of results.

Second, the sample size was relatively limited, which could affect the statistical power of the study and reduce the precision of generalizing the findings to a larger population.

Third, the study relied on measuring only two inflammatory markers (IL-6 and IL-8), whereas the immune response involves a broader spectrum of cytokines and other immune factors that were not assessed.

Fourth, other influential factors such as hormonal status, lifestyle, or general health status, which may play a significant role in explaining the variance in cytokine levels among individuals, were not thoroughly investigated.

Fifth, although the results indicated an association between bacterial type and cytokine levels as shown in the figure (Figure 2), the nature of the study (cross-sectional) does not allow for the establishment of a direct causal relationship between the variables.

Sixth, the severity of infection was not evaluated in detail clinically or correlated with clinical manifestations, which may limit the interpretation of the relationship between biomarkers and the actual disease state.

Ethical Considerations

Informed consent of participants was obtained prior to sample collection and confidentiality of information and its use for scientific research only was ensured. The study protocol was approved by the Institutional Research Ethics Committee of Peking University Health Science Center, Beijing, China, under approval number PKUHSC-REC-2025-041. Written informed consent was obtained from all participants prior to enrollment in accordance with the Declaration of Helsinki.

List of Abbrevations: **(IL-6):** interleukin-6; **(IL-8):** interleukin-8; **ELISA:** enzyme-linked immunosorbent assays; **PCR:** polymerase chain reaction; **SD:** standard deviation; **ROC:** Receiver operating characteristic; **AUC:** area under the curve.

Acknowledgment: This work was supported by grants from a consultancy project (2025-Na-24) of the research Institute of Public Health, Nankai University, (CAE).

Author Contribution: All authors contributed equally to the main contributor to this paper. All authors read and approved the final paper.

Funding: This work was supported by grants from a consultancy project (2025-Na-24) of the research Institute of Public Health, Nankai University, (CAE).

Conflicts of Interest: "The authors declare no conflict of interest."

References

[1] Brunham RC, Gottlieb SL, Paavonen J.(2015). Pelvic Inflammatory Disease. New England Journal of Medicine. 372(21):2039–2048. doi: https://doi.org/10.1056/NEJMra1411426

[2] Tanaka T, Narazaki M, Kishimoto T.(2014). IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology.6(10):a016295. doi: https://doi.org/10.1101/cshperspect.a016295

[3] Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. 2011. Vaginal Microbiome of Reproductive-Age Women. Proceedings of the National Academy of Sciences of the United States of America.108(Suppl 1):4680–4687. doi: https://doi.org/10.1073/pnas.1002611107

[4] Anahtar MN, Byrne EH, Doherty KE, Bowman BA, Yamamoto HS, Soumillon M, et al. 2015. Cervicovaginal Bacteria Are a Major Modulator of Host Inflammatory Responses in the Female Genital Tract. Immunity.42(5):965–976. doi: https://doi.org/10.1016/j.immuni.2015.04.019

[5] Kawai T, Akira S. 2010. The Role of Pattern-Recognition Receptors in Innate Immunity: Update on Toll-Like Receptors. Nature Immunology.11(5):373–384. doi: https://doi.org/10.1038/ni.1863

[6] Mitchell C, Balkus JE, Fredricks D, Liu C, McKernan-Mullin J, Frenkel LM, et al. 2015. Interaction Between Lactobacilli, Bacterial Vaginosis-Associated Bacteria, and Immune Mediators in the Female Genital Tract. Journal of Infectious Diseases. 210(12):1999–2007. doi: https://doi.org/10.1093/infdis/jiu302

[7] Baggiolini M. 1998. Chemokines and Leukocyte Traffic. Nature. 392(6676):565–568. doi: https://doi.org/10.1038/33340
[8] Masson L, Mlisana K, Little F, Werner L, Mkhize NN, Ronacher K, et al. 2015. Genital Inflammation and the Risk of HIV Acquisition in Women. Clinical Infectious Diseases.61(2):260–269. doi: https://doi.org/10.1093/cid/civ298
[9] Lequin RM. Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). Clinical Chemistry. 2005;51(12):2415–2418. doi: https://doi.org/10.1373/clinchem.2005.051532
[10] Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, et al. 2021. Sexually Transmitted Infections Treatment Guidelines. MMWR Recommendations and Reports.70(4):1–187. doi: https://doi.org/10.15585/mmwr.rr7004a1
[11] Peipert JF.2003. Clinical Practice: Genital Chlamydial Infections. New England Journal of Medicine.349(25):2424–2430. doi: https://doi.org/10.1056/NEJMcp030542
[12] Brotman RM. (2011). Vaginal Microbiome and Sexually Transmitted Infections: An Epidemiologic Perspective. Journal of Clinical Investigation. 121(12):4610–4617. doi: https://doi.org/10.1172/JCI57172