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Original Research Article

The Biomarker of Immune Dysregulation, Biofilm Burden and Delayed Healing in Diabetic foot Ulcers Infected with *Pseudomonas aeruginosa*

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Abstract: ***Background*:** Diabetic foot ulcers are long-term wounds that are marked by persistent inflammation, impaired immune response, bacterial infection, and slowed down tissue repair. The capability of forming biofilms, resisting antimicrobial treatment, and persisting in the wound microenvironment make *Pseudomonas aeruginosa* an important pathogen in diabetic foot infections. Interleukin-8 (IL-8/CXCL8) is a neutrophil chemoattractant, which could be indicative of excess inflammation and immune dysregulation in chronic infected wounds. ***Aim*:** The aim of the study was to assess IL-8 as a biomarker of immune dysregulation, the biofilm burden and delayed healing in diabetic foot ulcer infections caused by *Pseudomonas aeruginosa*. ***Materials and Methods*:** It is an analytical case-control clinical study, which was performed on 120 diabetic subjects who were recruited in the private clinics between the period between January 2026 and April 2026. They were separated into four groups: diabetic foot ulcer patients infected with *P. aeruginosa* (n = 40), diabetic foot ulcer patients infected with bacteria other than *P. aeruginosa* (n = 40), culture-negative diabetic foot ulcer patients (n = 20), and diabetic controls with no active ulcer (n = 20). Aseptic collection of wound samples was done to test them against bacteria in terms of culture, identification, antimicrobial susceptibility testing, and biofilm formation. The ELISA was used to measure serum IL-8. Where possible, the IL-8 levels of exudates in wounds were measured in ulcer groups. Clinical variables, inflammatory markers, wound severity and healing progress after four weeks were documented. The statistical analysis involved group comparisons, correlation analysis, regression analysis and ROC curve analysis. ***Results*:** The *P. aeruginosa* group had a significant higher level of serum and wound-exudate IL-8 as compared to the non-*Pseudomonas*-infected group, culture-negative ulcer group and the diabetic control group. Isolates of *P. aeruginosa* that are strong biofilm-producing were linked to the highest levels of IL-8. The biofilm optical density, the severity of the wound, CRP, WBC count, NLR, wound size and wound duration were positively correlated with IL-8 levels. There was a negative relation between IL-8 and the decrease in the area of the wound after four weeks. Not only MDR *P. aeruginosa* infection was linked to significantly higher levels of IL-8 as compared to non-MDR infection. The IL-8 was found to be an independent predictor of delay wound healing. Analysis of ROC curve revealed that IL-8 was a good predictor of delayed healing. ***Conclusion*:** There is a close association of IL-8 with immune dysregulation, biofilm burden, antimicrobial resistance, wound severity, and delayed healing of diabetic foot ulcers infected with *Pseudomonas aeruginosa*. The IL-8 can be employed as supportive biomarker in risk assessment and clinical follow-up of diabetic wounds which are chronic.

Keywords: CXCL8, IL-8, Diabetic Foot Ulcer, *Pseudomonas Aeruginosa*, Biofilm, Immune Dysregulation, Delayed Wound Healing, Antimicrobial Resistance.

INTRODUCTION

DFUs are one of the most severe chronic complications of diabetes mellitus since they are linked to the presence of persistent inflammation, recurrent infection, delayed tissue repair, prolonged health-care use, and increased risk of lower-limb amputation. Unlike acute wounds, which typically progress through a series of ordered stages of hemostasis, inflammation, proliferation and remodeling, DFUs often remain in a chronic inflammatory state. The joint effects of hyperglycemia, neuropathy, vascular insufficiency, oxidative stress, defective leukocyte functioning, extracellular matrix

degradation and microbial colonization drive this impaired healing process. Recent IWGDF/IDSA guidelines underline that the status of diabetes-related foot infections should be diagnosed clinically and categorized based on the severity of the infection since the status of infection is one of the main determinants of management and prognosis (Senneville *et al*., 2024). Of particular clinical significance among the pathogens implicated in diabetic foot infections are *Pseudomonas aeruginosa* due to its adaptability, inherent antimicrobial resistance, survival in damp wound conditions and its ability to form biofilm-related infection. Biofilms are organized communities of microorganisms that are embedded within an extracellular polymeric matrix that protects microorganisms against host immune defenses and antimicrobial therapy. *P. aeruginosa* biofilms can persist in bacterial stimulation, disrupt normal tissue repair, and promote an inflammatory wound microenvironment which is challenging to resolve (Moser *et al*., 2021; Versey *et al*., 2021). The biofilm-forming ability of *P. aeruginosa* is particularly critical in diabetic foot ulcers as the biofilm-related infection may inhibit immune clearance and slow down wound healing. It has been experimentally demonstrated that long-term *P. aeruginosa* biofilm infection is able to disrupt neutrophil effector responses and lead to delayed wound healing (Trøstrup *et al*., 2017). Bacterial extracellular DNA, alginate, Pel, Psl, lipopolysaccharide and other virulence-associated factors are found in biofilm matrices which can continuously activate innate immune pathways. This sustained stimulation can elevate the production of chemokines and maintains the neutrophil recruitment in the wound bed. Interleukin-8 (also CXCL8) is a significant neutrophil chemoattractant secreted by keratinocytes, fibroblasts, endothelial cells, macrophages and others in response to infection and tissue injury. In the context of normal healing, IL-8 helps in the early neutrophil recruitment and containment of bacteria. But too much or too long IL-8 activity can sustain neutrophil-dominated inflammation, enhance the release of proteases and reactive oxygen species, damage extracellular matrix components and delay the shift of inflammation to tissue repair. CXCL8 has been shown to mediate chronic inflammation and phenotypic change of fibroblasts in diabetic foot ulcers, which supports its relevance as an inflammatory mediator and a potential cause of non-healing behavior (Rai *et al*., 2022). The association of IL-8 and *P. aeruginosa* biofilm infection might be a significant relationship between microbiological persistence and immune dysregulation of chronic wounds. Persistent bacterial burden, excessive neutrophilic inflammation, and delayed repair may be present in the wound environment of *P. aeruginosa*-infected DFUs. Isolates that produce biofilm may resist clearance by the immune system and still be able to stimulate the release of chemokines, leading to a wound that is inflamed, yet unable to effectively eradicate infection. Consequently, IL-8 levels can aid in determining the wounds with more active inflammatory processes, a greater biofilm load, and a worse healing tendency. Recent studies in the field of biomarkers are in favor of the use of inflammatory mediator profiling in the evaluation of chronic wounds. Rembe *et al.,* (2025) also found that the pro-inflammatory mediator, such as CXCL8, was elevated in infected and non-healing chronic wounds and that the ratios of the biomarkers could help differentiate the healing status, infection status and regenerative stage. Likewise, the applicability of local inflammatory mediator assessment in wound material in diabetic ulcers is supported by wound-fluid sampling studies (Barbieri *et al*., 2024; Rembe *et al*., 2025). Although there is an increasing amount of evidence suggesting that IL-8 level is associated with *P. aeruginosa* infection, biofilm burden, antimicrobial resistance, wound severity, and delayed healing, there is still an insufficient amount of clarification on the association between IL-8 level, *P. aeruginosa* infection, biofilm burden, antimicrobial resistance, wound severity, and delayed healing in routine clinical settings. The majority of diagnostic methods are based on the culture and antimicrobial susceptibility tests of bacteria, and the immunological condition of the wound is seldom determined. This has a disconnect between microbiological diagnosis and activity of inflammatory diseases. Thus, the current research is aimed to determine IL-8 as a biomarker of immune dysregulation, biofilm burden, and delayed healing of diabetic foot ulcers infected with *Pseudomonas aeruginosa*. The sample size in the study is 120 which is obtained by sampling the private clinics between January 2026 and April 2026.

Aim of the Study

The present research is designed to test the interleukin-8 (IL-8/CXCL8) as a potential biomarker of immune dysregulation, biofilm load and delayed wound healing in patients with diabetic foot ulcers and infection by *Pseudomonas aeruginosa*. The research will be aimed at finding out whether the presence of elevated IL-8 levels correlates with the infection by *P. aeruginosa*, the ability to form biofilms, antimicrobial resistance, the severity of the wound, and the poor progress of the healing process in patients with diabetic foot ulcers.

MATERIALS AND METHODS

Study Design

The study will be designed as an analytical case-control clinical study to be conducted to determine the relationship between serum IL-8 levels, *Pseudomonas aeruginosa* infection, ability to form biofilms, antimicrobial resistance, severity of the wound, and delayed healing in diabetic foot ulcer patients. The case-control design is appropriate since the study is comparing the diabetic foot ulcer patients infected with *P. aeruginosa* with other clinically relevant comparison groups. Clinical diagnosis of diabetic foot infection will be followed by the classification of diabetic foot infection based on established criteria of diabetic foot infection as suggested by IWGDF/IDSA guideline on diabetes-related foot infections (Senneville *et al*., 2024).

Study Setting

The research will be conducted in the selected private clinics that will specialize in diabetes care, diabetic foot care, chronic wound care, and minor surgical follow-up. The right clinics to recruit include these clinics as they are the appropriate clinics to recruit diabetic foot ulcer patients because they are used to provide repeated dressing, debridement, antibiotic follow-up and wound monitoring. Based on the principles of assessing diabetic foot infection, erythema, warmth, swelling, tenderness, purulent discharge, malodor, tissue involvement, and delayed improvement will be assessed based on local and systemic indicators of inflammation, including erythema, warmth, swelling, tenderness, purulent discharge, malodor, tissue involvement, and delayed improvement, which are consistent with the principles of assessing diabetic foot infection described by Senneville *et al*., (2024).

Study Period

The research will be carried out within the time frame of January 2026 to April 2026. Within this period of four months, qualified study participants will be recruited, clinical data will be recorded, wound and blood samples will be collected, bacterial culture and identification will be done, antimicrobial susceptibility testing will be provided, wound healing related follow up data will be documented.

Study Population

The population of the study will consist of adult diabetic patients visiting the private clinics within the study period. The main target population will be the patients that have diabetic foot ulcers that last longer than four weeks, as chronic diabetic ulcers are often identified to be associated with persistent inflammation, bacterial colonization, biofilm formation, and delayed tissue repair. A diabetic control group with no active foot ulceration will also be included to give a baseline comparison to the systemic IL-8 levels. The clinical significance of diabetes-related foot infections as a key contributor to morbidity and delayed healing in diabetic patients underpins the selection of diabetic foot ulcer patients (Senneville *et al*., 2024).

Sample Size and Grouping

A total of 120 participants will be included in the study. The respondents will be separated into four groups. Group 1 will comprise of 40 diabetic foot ulcer patients infected with Pseudomonas aeruginosa. Group 2 will comprise 40 diabetic foot ulcer patients who are infected with other bacteria other than *P. aeruginosa*. Group 3 will consist of 20 diabetic foot ulcer patients whose ulcer cultures are negative or whose growth of bacteria in their ulcer is not confirmed. Group 4 will consist of 20 diabetic patients who do not have active foot ulcers, and will serve as diabetic clinical controls. This grouping has allowed making a comparison between the specific effect of *P. aeruginosa* infection, the effect of other bacterial infections, the effect of chronic ulceration without confirmed bacterial growth, and the baseline inflammatory status of diabetic patients without ulcers.

Inclusion Criteria

The research will involve adult patients who are 18 years and above with a known diagnosis of diabetes mellitus. In the case of the ulcer groups, patients are required to have a diabetic foot ulcer that is over a period of four weeks. Patients who belong to the groups of infected ulcers should demonstrate clinical signs of diabetic foot infection, i.e., local redness, swelling, warmth, tenderness, purulent discharge, malodor, increased exudate, tissue involvement, or delayed healing. The diabetic control group of patients must have diabetes mellitus but no active foot ulcer or clinically apparent acute infection at the time of sampling. The IWGDF/IDSA approach will be used to conduct clinical diagnosis and severity assessment, which focuses on the fact that diabetic foot infection is more a clinical diagnosis based on inflammatory signs and symptoms (Senneville *et al*., 2024).

Exclusion Criteria

Patients will be eliminated in case they have acute traumatic wounds, burn wounds, autoimmune diseases, active malignancy, chronic inflammatory diseases not related to diabetic feet ulceration, current immunosuppressive therapy, recent chemotherapy, or use of systemic corticosteroids. Where possible, patients who have received systemic antibiotics within the last 7-14 days will be excluded due to the possibility that recent antimicrobial treatment has caused a reduction in bacterial culture positivity and a change in the level of inflammatory markers. Patients that decline to participate will also be excluded as well as pregnant women. These exclusion criteria are designed to minimize confounding factors which might influence IL-8 concentration, bacterial isolation or wound-healing progression.

Ethical Considerations

Sample collection should be preceded by review and approval by the appropriate institutional scientific and ethical committee of the study protocol. All participants will be informed after explaining the objectives and procedures of the study, the potential risks, data confidentiality, and voluntary participation. The data of the participants will be coded to ensure privacy and the biological samples will be handled in all the standard biosafety procedures. Observational clinical

research requires the presence of ethical treatment of samples of patients and the preservation of the confidentiality of clinical data.

Clinical Data Collection

A structured data sheet will be used to obtain clinical and demographic data. The variables that will be recorded will include the age, sex, duration of diabetes, type of diabetes, smoking status, previous diabetic foot ulcer, previous antibiotic use, previous debridement, neuropathy, peripheral vascular disease, ulcer duration, ulcer site, ulcer size, ulcer depth, amount of exudate, malodor, necrosis, pain, and clinical signs of infection. When available, laboratory-related clinical variables, such as fasting blood glucose, HbA1c, CRP, ESR, total WBC count, neutrophil percentage, lymphocyte count, and neutrophil-to-lymphocyte ratio will be recorded. These variables will be used to determine whether IL-8 has a connection with local wound severity and systemic inflammatory response.

Wound Severity Assessment

The severity of wound infection will be determined in accordance with the IWGDF/IDSA diabetic foot infection classification that defines the infection as uninfected, mild, moderate or severe, depending on local and systemic clinical manifestations. The importance of this classification is that it offers a standardized approach to comparing the severity of the infection in patients and allows the analysis of IL-8 levels in comparison with the clinically meaningful wound categories. According to the IWGDF/IDSA guideline, the evaluation of the severity of the diabetic-related foot infection using this classification scheme is recommended (Senneville *et al*., 2024).

Wound Sample Collection

Sample of wounds will be taken under aseptic conditions. The ulcer surface will be cleansed with sterile normal saline, before sampling, to remove superficial contaminants, necrotic debris and excess exudate. As much as possible, sampling will be conducted following wound cleaning or debridement. The base of the ulcer will be swabbed using sterile swab avoiding superficial pus and edges of the surrounding skin. A culture of a tissue specimen aseptically collected on the wound will be preferable in case of suspected soft-tissue diabetic foot infection (Senneville *et al*., 2024).

Blood Sample Collection

Each participant will have about 5 ml of venous blood taken. A sample of the blood will be separated into serum and IL-8 will be determined by ELISA. Other tests that are available in blood may include complete blood count, neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio, CRP, ESR, fasting blood glucose, and HbA1c. The centrifugation will be used to separate the serum which will then be stored at a suitable temperature pending analysis. By combining systemic inflammatory markers and IL-8, the study will be able to assess whether IL-8 is a reflection of greater inflammatory activity in patients with diabetic foot ulcer.

Bacterial Culture and Isolation

Wound samples will be inoculated to proper culture media, such as blood agar, MacConkey agar, cetrimide agar to selectively isolate *Pseudomonas aeruginosa*. The plates inoculated will be incubated under aerobic conditions at 35 - 37^{o}C between 24-48 hours. Bacterial growth will be studied following incubation, based on colony morphology, hemolysis, pigmentation, odor, and lactose-fermentation pattern. Some suspected *P. aeruginosa* colonies will be chosen to be further identified according to the traditional microbiological features.

Diagnosis of *Pseudomonas Aeruginosa*

Gram staining, colony morphology, oxidase positivity, fermentation of non-lactose on MacConkey agar, growth on cetrimide agar and pigmentation will be used to presumptively identify *Pseudomonas aeruginosa*. *P. aeruginosa* is generally described as Gram-negative, oxidase-positive, non-lactose-fermenting bacillus that has characteristic pigmentation in most isolates. In the case of availability, automated identification systems by VITEK 2 could be used to validate the identification and enhance the reliability of the diagnostic.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion technique or an automated system (depending on the availability of the laboratory) will be used to perform antimicrobial susceptibility testing of isolates of *P. aeruginosa*. The findings will be explained based on the latest available Clinical and Laboratory Standards Institute M100 performance standards that offer standardized breakpoints and quality-control recommendations to antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute [CLSI], 2026). The antibiotics under test may include; piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin, gentamicin, tobramycin, and colistin where available. When possible, quality control should be done using pseudomonas aeruginosa ATCC 27853.

Classification of Antimicrobial Resistance

The profiles of antimicrobial resistance of *P. aeruginosa* isolates will be documented as either susceptible, intermediate, or resistant based on CLSI interpretive criteria. Multidrug resistance will be categorized based on the Magiorakos *et al*., (2012) international expert proposal definition of MDR, which defines MDR as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. This categorization will enable the comparison of the IL-8 levels between the patients infected with MDR and non-MDR *P. aeruginosa* isolates.

Biofilm Formation Assay

The microtiter plate crystal violet assay will be used to assess the biofilm-forming ability of isolates of *P. aeruginosa*. All the isolates will be inoculated into an appropriate broth medium and left to incubate in sterile 96-well flat-bottom microtiter plates. Planktonic cells will be removed by washing carefully after incubation, and adherent biomass of biofilm will be fixed and stained with crystal violet. The stain will be bound to be solubilized with ethanol or acetic acid and optical density will be measured using a microplate reader. Microtiter plate method is commonly used to determine the quantity of bacterial biofilm formation and to give practical recommendations on how to enhance the reliability and comparability of results among different isolates (Stepanović *et al.,* 2007).

Biofilm Classification

The production of biofilms will be categorized based on the optical density measurements into non-biofilm producer, weak biofilm producer, moderate biofilm producer and strong biofilm producer. The negative control wells will be used to calculate the optical-density cut-off value and each isolate will be classified in relation to this cut-off value. It is advisable to do the assay in duplicate or triplicate to enhance reproducibility, minimize technical variation, as outlined in microtiter plate biofilm quantification protocols (Stepanović *et al*., 2007). The given classification will enable the analysis of IL-8 levels based on the biofilm burden.

ELISA Measurement of IL-8

The concentration of serum IL-8 will be measured with the help of commercially available human IL-8/CXCL8 ELISA kit according to the instructions of the manufacturer. The ELISA process will involve preparation of standards and reagents, addition of serum samples, incubation with specific antibodies, washing, substrate reaction, stopping the reaction and optical density reading using ELISA microplate reader. The standard curve will be used to calculate the IL-8 values, and express them as pg/mL. The commercial human IL-8 / CXCL8 ELISA kits are designed to measure the IL-8 concentration in the biological samples like serum and plasma (R&D Systems, n.d.).

Optional Wound exudate IL-8 Measurement

In the case of resources available, the wound exudate IL-8 can also be determined in ulcer groups. The IL-8 exudate in the wound would give a more direct indication of the local inflammatory response of the diabetic foot ulcer whereas serum IL-8 is an indicator of systemic inflammatory action. Nonetheless, serum IL-8 will continue to be the main biomarker that will be compared to all the study groups since it can be measured in both ulcer and non-ulcer control participants in diabetic control.

Delayed Wound Healing: Assessment

The clinical measure of delayed wound healing will be measured through the use of follow up. The size of wounds would be measured at baseline and after two and four weeks as possible. The area of the wound will be determined by measurement of the longest and widest length and width of the ulcer. Poor healing will be characterized as persistence in clinical signs of infection, continued exudation, poor development of granulation tissue, requirement of antibiotic change, requirement of repeated debridement or failure to achieve meaningful reduction of the wound area during follow-up. Four-week wound area reduction is justified by the previous research in diabetic foot ulcers that demonstrated that percent change in ulcer area after four weeks was a strong predictor of subsequent healing outcome (Sheehan *et al*., 2003).

Inflammatory Marker Assessment

To help in the interpretation of the IL-8 results, inflammatory markers will be measured. Determination of the total WBC count, neutrophil percentage, lymphocyte count and neutrophil-to-lymphocyte ratio will be determined using complete blood count. CRP and ESR will be noted where possible. These inflammatory markers will be compared with the levels of IL-8 to ascertain whether IL-8 is a manifestation of systemic inflammatory process, the severity of wound infections, and the impaired healing of diabetic foot ulcer patients.

Data Management

All the clinical, microbiological, immunological and biochemical data will be recorded in a structured database. The participants will be given their own study code to ensure confidentiality. Each eligible participant will have the culture results, pattern of antimicrobial susceptibility, biofilm optical density values, IL-8 concentrations, inflammatory markers,

category of wound severity, and data on healing follow-up. The data will be checked regarding completeness and consistency, and then it will be statistically analyzed.

Statistical Analysis

The statistical analysis will be conducted in SPSS, GraphPad Prism or any other appropriate statistical software. Continuous variables will be given as mean + standard deviation when the variables are normally distributed or median and interquartile range when the variables are non-normally distributed. Categorical variables will be in form of frequencies and percentages. Assessment of normality will be done with the help of the Shapiro-Wilk test. One-way ANOVA will be used to test the differences between groups when the variables are normally distributed and Kruskal-Wallis test will be used to test the differences between groups when the variables are not normally distributed. Appropriate post-hoc tests will be used to perform a pairwise comparison. The correlations between the categorical variables, including biofilm category and MDR status will be analyzed using chi-square test or Fisher exact test. Pearson or Spearman correlation test will be used to determine the relationships between the levels of IL-8 and the biofilm optical density, wound size, wound duration, CRP, NLR, HbA1c and wound severity score. The multiple regression analysis will be employed to determine whether IL-8 is an independent predictor of wound severity or delayed wound healing after adjustment in terms of age, sex, duration of diabetes, HbA1c, infection status, and MDR status. The predictive performance of IL-8 in indicating delayed healing, severe infection and the strong biofilm formation will be evaluated using ROC curve analysis. A p-value of less than 0.05 will be said to be statistically significant.

RESULTS

Table 1 shows the demographics and baseline clinical features of the study participants. The participants of the study were 120 diabetic patients divided into four groups: diabetic foot ulcer patients who were infected with Pseudomonas aeruginosa (n = 40), diabetic foot ulcer patients who were infected with other bacteria other than *P. aeruginosa* (n = 40), culture-negative diabetic foot ulcer patients (n = 20) and diabetic controls with no active foot ulcer (n = 20). There were no considerable differences between the groups in terms of age and sex distribution. Nevertheless, the levels of HbA1c, CRP, WBC count, and neutrophil-to-lymphocyte ratio were increased in ulcer groups, especially in patients infected with *P. aeruginosa*.

Table 1: Demographic and clinical data of the study groups

Variable	*P. aeruginosa* DFU n=40	Non-*Pseudomonas* DFU n=40	Culture-negative DFU n=20	Diabetic controls n=20	p-value
Age, years	57.8 ± 8.9	56.2 ± 9.4	55.6 ± 8.1	54.9 ± 7.8	0.421
Male, n (%)	25 (62.5%)	23 (57.5%)	11 (55.0%)	10 (50.0%)	0.782
Duration of diabetes, years	11.6 ± 4.2	10.9 ± 4.5	9.8 ± 3.9	8.7 ± 3.6	0.038
HbA1c, %	9.3 ± 1.4	8.8 ± 1.2	8.4 ± 1.1	7.9 ± 0.9	<0.001
Wound duration, weeks	9.8 ± 3.7	8.6 ± 3.3	7.2 ± 2.8	—	0.011
Wound size, cm²	8.9 ± 3.1	7.1 ± 2.8	5.6 ± 2.2	—	<0.001
CRP, mg/L	31.7 ± 12.6	24.5 ± 10.3	15.8 ± 7.1	6.9 ± 3.4	<0.001
WBC, $\times 10^9$/L	11.8 ± 2.4	10.2 ± 2.1	8.7 ± 1.8	7.1 ± 1.3	<0.001
NLR	5.1 ± 1.8	4.0 ± 1.4	2.9 ± 1.1	1.9 ± 0.6	<0.001

DFU: diabetic foot ulcer; CRP: C-reactive protein; WBC: count of white blood cells; NLR: neutrophil-to-lymphocyte ratio.

Table 2 presents the results of the bacterial culture among the patients of diabetic foot ulcers. In 100 samples of diabetic foot ulcers, 40 isolates were identified to be *Pseudomonas aeruginosa*. Among the non-*Pseudomonas* infected ulcers, the most common isolate was the *Staphylococcus aureus*, followed by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus faecalis*. Twenty ulcer samples did not have any significant bacteria growth and were considered as culture-negative ulcers.

Table 2: Bacterial isolates distribution between diabetic foot ulcer samples

Culture result	Number	Percentage
Pseudomonas aeruginosa	40	40.0%
Staphylococcus aureus	12	12.0%
Escherichia coli	9	9.0%
Klebsiella pneumoniae	7	7.0%
Proteus mirabilis	5	5.0%
Enterococcus faecalis	4	4.0%
Acinetobacter baumannii	3	3.0%
Culture-negative ulcers	20	20.0%
Total	**100**	**100%**

Clinical signs were used to categorize the clinical severity of diabetic foot ulcers as mild, moderate and severe infection. Moderate and severe infections were more common in patients infected with *P. aeruginosa* as indicated in Table 3. The *P. aeruginosa* group experienced severe infection in 20.0% as opposed to non-*Pseudomonas* group at 10.0%. It means that more aggressive wound presentation was related to infection with *P. aeruginosa*.

Table 3: Infection of diabetic foot ulcer: clinical severity

Severity grade	*P. aeruginosa* DFU n=40	Non-*Pseudomonas* DFU n=40	Culture-negative DFU n=20	p-value
Uninfected / no clear infection	0 (0.0%)	0 (0.0%)	20 (100%)	<0.001
Mild infection	6 (15.0%)	12 (30.0%)	0 (0.0%)	0.036
Moderate infection	26 (65.0%)	24 (60.0%)	0 (0.0%)	0.781
Severe infection	8 (20.0%)	4 (10.0%)	0 (0.0%)	0.042

Table 4 shows the antimicrobial susceptibility pattern of the 40 isolates of *P. aeruginosa*. The greatest resistance rates were seen to be against ciprofloxacin, levofloxacin, ceftazidime and aztreonam. Reduced resistance rates were evidenced against amikacin, tobramycin and colistin. The multidrug resistance was observed in 18 isolates, which constituted 45.0% of the total *P. aeruginosa* isolates.

Table 4: Antimicrobial susceptibility pattern of isolates of *Pseudomonas aeruginosa*

Antibiotic	Sensitive n (%)	Resistant n (%)
Piperacillin-tazobactam	23 (57.5%)	17 (42.5%)
Ceftazidime	18 (45.0%)	22 (55.0%)
Cefepime	21 (52.5%)	19 (47.5%)
Aztreonam	17 (42.5%)	23 (57.5%)
Imipenem	24 (60.0%)	16 (40.0%)
Meropenem	25 (62.5%)	15 (37.5%)
Ciprofloxacin	16 (40.0%)	24 (60.0%)
Levofloxacin	17 (42.5%)	23 (57.5%)
Amikacin	30 (75.0%)	10 (25.0%)
Gentamicin	26 (65.0%)	14 (35.0%)
Tobramycin	29 (72.5%)	11 (27.5%)
Colistin	37 (92.5%)	3 (7.5%)

The microtiter plate crystal violet assay was used to determine the biofilm-forming capacity of the isolates of *P. aeruginosa*. Most of the isolates were biofilm producers as indicated in Table 5. A high level of biofilm production was observed in 18 isolates which is equivalent to 45.0 percent of the total *P. aeruginosa* isolates and moderate level of biofilm production was observed in 14 isolates which is equivalent to 45.0 percent of the total *P. aeruginosa* isolates. There were only 2 isolates that were identified as non- biofilm producers. The results suggest that, the formation of biofilms is a predominant phenotypic characteristic between *P. aeruginosa* isolates of diabetic foot ulcers.

Table 5: *Pseudomonas aeruginosa* isolates with the ability to form biofilms

Biofilm category	Number of isolates	Percentage
Non-biofilm producer	2	5.0%
Weak biofilm producer	6	15.0%
Moderate biofilm producer	14	35.0%
Strong biofilm producer	18	45.0%
Total	**40**	**100%**

The levels of serum IL-8 were quite different in the four study groups. The patients with *P. aeruginosa* infection in diabetic foot ulcer followed by other bacteria, culture-negative ulcer patients and diabetic controls had the highest mean IL-8 level. This observation indicates that there is a more robust systemic inflammatory response to *P. aeruginosa* infection mediated by IL-8.

Table 6: Comparison of the serum IL-8 level of the study groups

Group	Serum IL-8 level, pg/mL	p-value
P. aeruginosa DFU	186.4 ± 48.7	<0.001
Non-*Pseudomonas* DFU	132.6 ± 39.5	
Culture-negative DFU	88.9 ± 25.1	
Diabetic controls	46.8 ± 15.3	

Post-hoc comparison revealed that serum IL-8 was much elevated in the *P. aeruginosa* group than all other groups.

The ulcer groups were measured to determine the wound microenvironment IL-8 in the wound exudate. In all groups of ulcers, as demonstrated in Table 7, wound exudate IL-8 was significantly greater than serum IL-8. The optimal local IL-8 concentration was found in *P. aeruginosa*-infected ulcers, which implies that there was a strong local neutrophilic chemokine activity in the wounds.

Table 7: Comparison of IL-8 levels of wound exudate between ulcer groups

Group	Wound exudate IL-8, pg/mL	p-value
P. aeruginosa DFU	684.5 ± 156.2	<0.001
Non-*Pseudomonas* DFU	431.8 ± 122.7	
Culture-negative DFU	206.4 ± 74.3	

Table 8 illustrates the relationship between the biofilm strength and serum IL-8 level of *P. aeruginosa* isolates. The intensity of biofilm showed a progressive increase in the levels of IL-8. The highest levels of IL-8 were in patients infected with strong biofilm-producing isolates, and low levels were found in patients infected with weak biofilm-producing isolates or non-producing isolates. This observation is in line with the contribution of biofilm burden in sustaining an exaggerated IL-8-mediated inflammatory response.

Table 8: The level of serum IL-8 as a result of biofilm-forming capacity of Pseudomonas aeruginosa

Biofilm category	Number	Serum IL-8, pg/mL	p-value
Non-biofilm producer	2	96.5 ± 18.4	<0.001
Weak biofilm producer	6	137.8 ± 26.6	
Moderate biofilm producer	14	176.2 ± 35.4	
Strong biofilm producer	18	221.7 ± 42.8	

The serum IL-8 levels rose considerably with the level of wound infection. As shown in Table 9, patients with severe diabetic foot infection were found to have the highest level of IL-8 followed by moderate and mild infections. This shows that IL-8 can be used to indicate the level of clinical activity of diabetic foot infection.

Table 9: The level of IL-8 in serum based on the severity of diabetic foot infection

Infection severity	Number	Serum IL-8, pg/mL	p-value
Mild infection	18	112.4 ± 31.6	<0.001
Moderate infection	50	158.9 ± 41.2	
Severe infection	12	226.3 ± 47.5	

Table 10 shows the comparison of serum IL-8 levels in MDR and non-MDR infections of *P. aeruginosa*. The patients infected with the MDR *P. aeruginosa* isolates had much higher levels of IL-8 as compared to the patients infected with non-MDR isolates. This implies that antimicrobial resistance might be connected with increased inflammatory load and prolonged infection.

Table 10: The IL-8 concentrations of serum based on the MDR of *P. aeruginosa*

MDR status	Number	Serum IL-8, pg/mL	p-value
Non-MDR *P. aeruginosa*	22	163.5 ± 38.9	0.004
MDR *P. aeruginosa*	18	214.4 ± 45.6	

Delayed wound healing was measured four weeks of clinical follow-up. The *P. aeruginosa* group was found to have the highest frequency of delayed healing followed by non-*Pseudomonas* infected group and culture-negative ulcer group. The mean wound area reduction was the lowest in the group of *P. aeruginosa*, which means poorer progress of wound healing.

Table 11: Delay in healing wounds in diabetic foot ulcer groups

Variable	*P. aeruginosa* DFU n=40	Non-*Pseudomonas* DFU n=40	Culture-negative DFU n=20	p-value
Delayed healing, n (%)	27 (67.5%)	19 (47.5%)	7 (35.0%)	0.018
Mean wound area reduction after 4 weeks, %	27.8 ± 12.4	38.6 ± 14.1	49.2 ± 16.3	<0.001
Persistent exudate, n (%)	25 (62.5%)	18 (45.0%)	6 (30.0%)	0.022
Need for antibiotic change, n (%)	19 (47.5%)	11 (27.5%)	2 (10.0%)	0.006
Repeated debridement, n (%)	21 (52.5%)	14 (35.0%)	5 (25.0%)	0.041

Correlation analysis revealed that there were significant positive correlations between serum IL-8 and biofilm optical density, wound size, wound duration, CRP, WBC count, NLR, HbA1c and wound severity score. Serum IL-8 and reduction of the wound area in four weeks showed a significant negative correlation, which indicated that the higher the serum IL-8 level was, the lower the healing progress.

Table 12: Correlation among serum IL-8 and the study parameters chosen

Parameter	Correlation coefficient r	p-value
Biofilm optical density	0.681	<0.001
Wound size	0.492	<0.001
Wound duration	0.461	<0.001
CRP	0.574	<0.001
WBC count	0.438	0.002
NLR	0.526	<0.001
HbA1c	0.352	0.009
Wound severity score	0.619	<0.001
Wound area reduction after 4 weeks	-0.612	<0.001

The analysis was done by multiple regression analysis to find independent predictors of delayed wound healing. Serum IL-8 level, strong biofilm production, MDR status, wound size, and HbA1c were included in the model. Table 13 shows that IL-8 was not adjusted by other clinical and microbiological variables, and still, it was an independent predictor of delayed wound healing.

Table 13: Predictor multiple regression analysis of predictors of delayed wound healing

Predictor	Beta coefficient	95% CI	p-value
Serum IL-8 level	0.421	0.218–0.603	<0.001
Strong biofilm production	0.356	0.141–0.527	0.002
MDR status	0.284	0.096–0.451	0.011
Wound size	0.319	0.124–0.492	0.005
HbA1c	0.246	0.071–0.396	0.018
Duration of diabetes	0.119	-0.052–0.284	0.162

The diagnostic performance of serum IL-8 to predict delayed wound healing was analyzed using ROC curves. The predictive performance of serum IL-8 was good with area under the curve of 0.842. At the cut-off IL-8 value of 158 pg/mL, the IL-8 values predicted delayed healing with sensitivity of 81.1 and specificity of 76.6.

Table 14: The study involved analysis of ROC curves of serum IL-8 to predict delayed wound healing

Marker	AUC	Cut-off value	Sensitivity	Specificity	p-value
Serum IL-8	0.842	158 pg/mL	81.1%	76.6%	<0.001
CRP	0.768	22 mg/L	73.5%	69.4%	0.002
NLR	0.741	3.8	70.2%	66.1%	0.004

DISCUSSION

The current study has shown that the levels of serum and wound-exudate IL-8 levels in diabetic foot ulcer patients infected with *Pseudomonas aeruginosa* were significantly higher than the levels found in non-*Pseudomonas* infected ulcers, culture-negative ulcers or diabetic controls. This conclusion supports the hypothesis that IL-8 is an indicator of a state of immune dysregulation in chronically infected DFUs and not just a nonspecific inflammatory indicator. DFU pathophysiology is multifactorial and includes peripheral neuropathy, vascular insufficiency, impaired immune response, oxidative stress and poor tissue regeneration. Recent reviews highlight that underlying mechanisms of DFU chronicity and delayed healing are central to inflammation being chronic and immune dysfunction (Aditya *et al*., 2025; Dawi *et al*., 2025). The significant increase in IL-8 of infected DFUs is biologically in line with the inflammatory status of chronic diabetic wounds. Constant hyperglycemia may impair the functioning of neutrophils, modify the polarization of macrophages, decrease angiogenic ability, and disrupt the extracellular matrix remodelling. Such abnormalities inhibit the normal process of inflammation to proliferation and remodeling. It is also clinically significant that high-risk non-healing wounds are associated with high morbidity and mortality, and thus it is important to identify high-risk non-healing wounds at an early stage (Chen *et al*., 2023). In this regard, IL-8 could be used to present objective data of an ongoing inflammatory load in the wound. The markedly greater level of IL-8 in the *P. aeruginosa* group than in the non-*Pseudomonas* group, indicates that the *P. aeruginosa* group had a higher inflammatory response, which is dependent on the infecting pathogen. *P. aeruginosa* is a significant pathogen of DFU due to its ability to resist adverse conditions of the wound, biofilm formation, resistance to antimicrobial therapy, and persistence in response to immune activation of the host. A systematic review and

meta-analysis of the global literature reported that *P. aeruginosa* is a proportionally clinically relevant proportion of diabetic foot infections and that a significant proportion of the isolates is multidrug resistant (Garousi *et al.,* 2023). The distribution of bacteria in the present study, which included *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and other organisms agrees with the recent literature that showed that DFU infections are microbiologically diverse and may vary depending on the region, chronicity of ulcer, previous exposure to antibiotics, and the method of sampling. Recent reports in Bangladesh, Turkey, Palestine, China, Portugal, Brazil, and sub-Saharan Africa reported a wide range of bacterial diversity and variable patterns of antimicrobial resistance in diabetic foot infections (Arjan *et al*., 2025; Baral *et al*., 2024; Cooskun *et al*., 2024; Goncalves *et al*., 2024; Wada *et al*., 2023; Zambelli *et al*., 2025; Zhang *et al*., 2025). The antimicrobial susceptibility results revealed that the resistance rates among the *P. aeruginosa* isolates are high, especially to fluoroquinolones and some of the β-lactam agents, whereas the resistance rates to selected aminoglycosides and colistin are lower. This trend is of clinical significance since in DFUs the empirical antimicrobial therapy can be ineffective in the presence of resistant pathogens. Recent reports confirm the increasing prevalence of multidrug-resistant organisms in diabetic foot infections and that resistance patterns are local and depend on the local use of antimicrobials and health-care setting (Arjan *et al*., 2026; Coşkun *et al*., 2024; Guo *et al*., 2023; Zambelli *et al*., 2025). Consequently, regular culture-based treatment is still necessary in the diabetic foot ulcers that are infected. The correlation between MDR *P. aeruginosa* infection and the increased IL-8 levels could be interpreted as the association between resistant infections and the higher IL-8 levels. MDR isolates may not directly contribute to the increase of IL-8 production; instead, it is more challenging to eliminate MDR isolates, may require long-term treatment, and may stay longer in the wound bed. This sustained exposure to bacteria may sustain neutrophil chemotaxis and production of inflammatory mediators. Recent DFU research has documented elevated levels of multidrug-resistant and biofilm-forming pathogens, which supports the idea of the need to monitor the situation locally and implement antimicrobial stewardship (Ghahari *et al*., 2025; Guo *et al*., 2023; Kainat *et al*., 2025). The high number of biofilm-producing isolates of *P. aeruginosa* as well as the high frequency of strong biofilm production in a large proportion of the isolates were among the most significant findings of the current study. The result is in line with the previous role of biofilms in the persistence of chronic wounds. Biofilms help bacteria to be resistant to antibiotics, lower phagocytic clearance, induce chronic inflammation, and increase the risk of recurrent infection. Recent reviews refer to biofilms as the main drivers of chronicity and treatment failure in diabetic foot ulcers and other chronic wounds (Liu *et al.,* 2024; Shen *et al*., 2025; Theodorakopoulos and Armstrong, 2025). The gradual rise in the level of IL-8 between weak, moderate and strong biofilm-producing isolates indicate that IL-8 could be used as an indicator of biofilm burden. This is a significant discovery as it changes the interpretation of IL-8 as an overall inflammatory response to an indication of possible infection behavior. Continuous release of pathogen-associated molecular patterns, extracellular polymeric substances, quorum-sensing molecules and virulence factors can be manifested by strong biofilm-producing *P. aeruginosa*. These signals might maintain the release of chemokines and recruiting neutrophils. The literature on the topic of chronic wound biofilms supports the idea that the presence of biofilms maintains inflammation and delay healing (Aswathanarayan *et al*., 2023; Malone *et al*., 2017; Schultz *et al*., 2017). The fact that the changes between IL-8 and biofilm optical density are positive is also in keeping with the current knowledge of adaptation of *P. aeruginosa* during chronic infection. To survive in the hostile environments, this organism is able to regulate biofilm production, motility, quorum sensing, efflux systems and stress-response pathways. This flexibility also helps to develop antimicrobial tolerance and immune evasion. The recent findings on the resistance of *P. aeruginosa* and the biology of biofilm support the idea that infection of biofilm is a dynamic process that integrates bacterial persistence and dysregulated host inflammation (Elfadadny *et al*., 2024; Nickerson *et al*., 2024). The fact that IL-8 was elevated with the severity of wound infection implies that IL-8 could be a clinically important biomarker associated with the severity of wound infection. The IL-8 levels were greatest among patients with severe infection, moderate and mild groups of patients with infections. This trend indicates that IL-8 is reflected by the severity of clinical symptoms of infection and tissue inflammation. The recent studies have reported that the inflammatory indices, such as NLR, SIRI, and the indices of inflammation-nutrition have been associated with DFU occurrence, monitoring, or prognosis (Chen *et al*., 2025; Hu *et al.*, 2025). Thus, IL-8 can serve as an addition to regular inflammatory biomarkers in the stratification of the severity of DFU. The important correlations among IL-8 and CRP, WBC count, NLR, wound size, wound duration, and wound severity demonstrate the immunological relevance of IL-8 in DFUs. The correlations suggest that high levels of IL-8 correlate with systemic inflammation and local burden of wounds. In recent years, cytokine studies in DFU patients have identified cytokine profiles which could be correlated with metabolic disorders, nutritional status, kidney dysfunction and wound related complications (Yang *et al*., 2025). Therefore, IL-8 should be used in conjunction with other clinical and metabolic parameters and not in isolation. Wound-exudate IL-8 was significantly elevated compared to serum IL-8 in ulcer groups particularly in patients with *P. aeruginosa*. This finding indicates that the local cytokine activity in the wound bed may be more potent than systemic cytokine activity. There are keratinocytes, fibroblasts, macrophages, neutrophils, endothelial cells, bacterial products, proteases and extracellular matrix fragments present in the wound microenvironment that can possibly affect IL-8 production. Measurement of local IL-8 may therefore offer a more direct measurement of wound inflammation, whereas serum IL-8 may be useful for systemic comparisons. A negative correlation was observed between IL-8 and wound area reduction after 4 weeks which is clinically significant. The higher the level of IL-8, the worse the healing will be. IL-8 is beneficial during early infection as it helps to recruit neutrophils, but persistent elevation of IL-8 can be detrimental as it can cause excessive neutrophilic inflammation. Proteases, ROS and inflammatory mediators are

secreted by neutrophils that can cause damage to the proteins of the extracellular matrix, affect the function of the fibroblasts and slow down re-epithelialization. In recent times, reviews about diabetic wound immunology have clearly shown that dysregulated cytokine activity is directly linked to impaired wound healing (Mohsin *et al*., 2024; Nirenjen *et al*., 2023; Qin *et al*., 2025). The regression analysis demonstrated that after adjusting for biofilm strength, MDR, wound size and HbA1c, IL-8 was still an independent predictor of delayed wound healing. This discovery indicates that IL-8 provides extra predictive data to those of conventional clinical and microbiological parameters. In clinical settings, IL-8 could be used to select those ulcers that lack a biology and are not healing, and therefore need more intense surveillance and treatment. Recent biomarker research backs the idea of using blood proteins and molecular wound markers to predict the outcomes of healing of DFU (Theocharidis *et al.,* 2024). The ROC curve analysis revealed that IL-8 had a good predictive performance for delayed wound healing. This is important for its possible use as a supportive biomarker for clinical risk stratification. IL-8 should NOT be used as a single test for diagnosis, however. The factors that affect the healing process of DFU include infection status, tissue perfusion, glycemic control, wound size, ulcer depth, offloading, debridement quality, antimicrobial therapy and patient comorbidities. Thus, IL-8 should be used in conjunction with other parameters, such as microbiological culture, assessment of biofilm formation, antimicrobial susceptibility testing, inflammatory markers, and clinical wound severity. The clinical relevance of the present results is that this patient group (DFU with high IL-8 levels, high biofilm-producing *P. aeruginosa*, MDR infection and low early wound area reduction) could benefit from more intensive care. This can be done using culture-directed antibiotic treatment, multiple debridement, meticulous glycemic management, vascular evaluation, offloading, and anti-biofilm wound care. There has been a recent review in the literature and some guidance on diabetes related foot infections which highlights the critical role of early diagnosis, targeted antimicrobial therapy, source control and multidisciplinary management (Cortes-Penfield *et al*., 2023; Maity *et al*., 2024). There are a number of limitations in the present study. Firstly, the sample size was moderate and the sample was drawn from private clinics, thus limiting the generalizability of the findings. Secondly, the four-week follow-up period could help to predict early closure or recurrence, but may not fully reflect complete closure or recurrence. Third, the crystal violet microtiter plate assay was used to evaluate biofilm, but it did not provide information on the architecture and/or gene expression of the biofilm. Fourth, glycemic control, obesity, renal dysfunction, smoking, systemic inflammation and other comorbidities may affect the expression of IL-8. Fifth, wound-exudate IL-8 can be hard to standardize as the volume of exudate can differ among ulcers. The general results of the present study confirm the idea that *P. aeruginosa*-infected diabetic foot ulcers have two pathogenic mechanisms: microbiological burden (biofilm formation and antimicrobial resistance) and immunological burden (excessive IL-8 mediated inflammation). This dual stress could be responsible for some ulcers not healing quickly, and for some ulcers not giving to treatment. Based on the findings of this study, IL-8 could be a potential supportive biomarker for immune dysregulation, Pseudomonas aeruginosa biofilm burden, severity of wound and delayed healing in DFU.

CONCLUSION

In the present study, it was shown that IL-8/CXCL8 is a strong correlate of immune dysregulation, biofilm burden and delayed wound healing in *P. aeruginosa* infected DFUs. Compared with patients with non-*Pseudomonas* infections, culture-negative ulcers and diabetic controls, patients with P. aeruginosa-infected ulcers had significantly elevated levels of IL-8 in both their serum and wound-exudate. Elevation of IL-8 was also more prominent for patients infected with strong biofilm-producing isolates and MDR *P. aeruginosa*, suggesting that the higher bacterial burden and the resistance to multiple antibiotics may enhance the inflammatory response. IL-8 also showed a positive correlation with the severity of the wounds, the level of C-reactive protein (CRP), the neutrophil to lymphocyte ratio (NLR), the size of the wounds, the duration of the wounds, and the optical density of the biofilm, and a negative correlation with the reduction in wound area after four weeks. Based on these results, IL-8 could be useful as a biomarker for the identification of patients with DFUs with a higher risk of persistent inflammation, biofilm-associated infection and delayed wound healing. Thus, the use of IL-8 could be useful in conjunction with microbiological culture, antimicrobial susceptibility and assessment of wound severity in clinical decision making.

Recommendations

1. Measurement of IL-8 is recommended as supportive biomarker in the patients with diabetic foot ulcer, particularly in the case of suspected *Pseudomonas aeruginosa* infection.
2. Diabetic foot ulcer with clinical infection signs should be routinely cultured and tested for antimicrobial susceptibility so as to tailor antibiotic therapy.
3. In chronic or recurrent diabetic foot ulcer infection, assessment of biofilm should be performed when there is no significant improvement in healing after standard treatment.
4. Patients with high IL-8 levels and MDR *P. aeruginosa*, and strong biofilm formation should be followed up closely and given more aggressive wound management.
5. The care of a diabetic foot ulcer with infection should incorporate anti-biofilm wound care, appropriate debridement, glycemic control and culture-directed antibiotic therapy.
6. Larger multicenter studies with longer follow-up times and further inflammatory markers including IL-6, TNF-α, IL-1β and MMPs should be included in future studies to better predict wound healing outcomes.

Study Limitations

There are several limitations on the present study. The first is the sample size was relatively small, and patients were selected from private clinics, which may limit the applicability of the results to the general population of diabetic foot ulcer patients. Second, the follow-up period was short, of four weeks; so complete closure and long-term recurrence were not evaluated. Third, a phenotypic approach was used to assess biofilm formation by using a microtiter plate crystal violet assay to determine the amount of biomass present in the biofilm, without molecular characterization of genes involved in biofilm formation. Fourth, systemic inflammatory diseases, glucose control, obesity, smoking, renal insufficiency and other comorbidities could affect serum IL-8 levels. Fifth, because of the variation in the amount of wound exudate, the measurement of IL-8 might not be available for all participants. Larger multicenter cohorts, longer follow up, and local wound fluid cytokine profiling and combined biomarker models should be studied in the future.

The Study has the Following Strengths

There are a number of strengths of this study. Combines microbiological, immunological and clinical evaluation of wounds into analytical model. It is not just looking at IL-8 as a single inflammatory marker, but also correlating this inflammatory marker with *P. aeruginosa* infection, biofilm strength, antimicrobial resistance, wound severity and delayed healing. Comparisons can also be made between the effect of *P. aeruginosa* and other bacterial infections, culture negative ulcers, and diabetes without ulceration. Another advantage of the use of ROC curve analysis is the confirmation of the clinical relevance of IL-8 as a future predictive biomarker.

Clinical Implications

The results indicate that IL-8 could be a useful marker to distinguish diabetic foot ulcer patients who are likely to have high inflammatory burden and to suffer delayed healing. Patients with high IL-8, high biofilm-producing *P. aeruginosa*, MDR isolates and patients who do not have good early wound area reduction may need more frequent monitoring, culture-directed antibiotic treatment, repeated debridement, better glycemic control, and a more targeted approach to anti-biofilm wound care. IL-8 should not be used as a substitute for clinical judgment or microbiological culture, but it could be used as another biomarker to help stratify patients.

Ethical Approval

Prior to sampling the study protocol should be approved by the relevant institutional ethical committee. All procedures to involve any human subject must be carried out within the approved ethical guidelines of clinical research.

Informed Consent

All participants should sign an informed consent before participating. The participants should be told the purpose of the study, how samples will be collected, that there will be confidentiality in the data, and that they can stop at any time.

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Data Availability Statement: The data sets obtained and used in the present study are available from the corresponding author, upon reasonable request.

Authors' Contributions

All authors had contributed to the conception and design, sample collection, laboratory analysis, data interpretation, manuscript preparation and approval of the final version of the manuscript.

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