



## Microbiological characteristics of ulcerative lesions in t2dm with consideration of the Wagner classification model

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**Abstract.** According to monitoring studies, the number of patients with type 2 diabetes mellitus among the population of Ukraine increases annually by an average of 5–7 %, followed by the development of Diabetic Foot Syndrome, the pathogenesis of which is caused by massive microbial contamination. A comprehensive approach to the diagnosis and treatment of this pathology, incorporating both the microbiological aspect and the use of various classification scales, will facilitate the standardization of clinical assessment and simplify the selection of therapeutic strategies. Objective of the study: to determine the relationship between the severity of purulent-necrotic lesions in Diabetic Foot Syndrome in patients with Type 2 Diabetes Mellitus and the degree of microbial contamination according to the Wagner classification. The study included wound exudate obtained from 75 patients with Type 2 Diabetes Mellitus complicated by Diabetic Foot Syndrome. The severity of soft tissue destruction was assessed by stratifying patients using the Wagner classification. Microbiological analysis was performed at the Department of Microbiology, Virology, and Immunology of I. Horbachevsky Ternopil National Medical University. The study findings demonstrated that the peak incidence of Diabetic Foot Syndrome was observed in the 61–70 age group: 34.6 % among men and 13.3 % among women. Assessment of the degree of carbohydrate metabolism compensation revealed a predominance of the subcompensation stage (78.6 %). According to the Wagner classification, grade IV lesions were predominant (82.7 %), in which strains of *Klebsiella* spp. and *Pseudomonas aeruginosa* were identified. According to the Wagner classification, a high frequency (82.7 %) of grade IV lesions was established, with a predominance of deep purulent-necrotic lesions associated with the presence of antibiotic-resistant strains of *Klebsiella* spp. and *Pseudomonas aeruginosa*.

**Keywords:** Diabetes Mellitus; Diabetic Foot Syndrome; microorganisms; Wagner classification; microbiota.

### Introduction

Diabetic Foot Syndrome (DFS) is a multifactorial complication of Type 2 Diabetes Mellitus (T2DM) that combines an infectious process, impaired perfusion, immune dysregulation, and progressive

tissue destruction, and requires radical approaches to the debridement of purulent-necrotic foci [1–3]. The development of this complication is closely associated with both microvascular and macrovascular complications, as well as microbial contamination.

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Microbiological analysis of wound exudate in patients with Diabetic Foot Syndrome allows for the identification of complex microbial associations, in which Gram-positive cocci have traditionally played a leading role. However, recently there has been a clear trend toward an increasing proportion of Gram-negative bacteria, particularly members of the family *Enterobacteriaceae*, in the structure of pathogens. Studies of the microbiome of poorly healing ulcers indicate the presence of specific microbial associations that suppress local immunity. As a rule, polymicrobial infections are more frequently observed in chronic cases that have previously been treated with antibiotics [4–6].

Particular concern is posed by staphylococci and representatives of the genus *Klebsiella*, which often act as dominant components of microbial communities. The prevalence of *Klebsiella pneumoniae* in diabetic foot infections is one of the significant challenges requiring the development of new therapeutic strategies. In addition to typical pathogens, rare microorganisms are increasingly being identified in the wounds of patients with Type 2 Diabetes Mellitus, such as *Raoultella ornithinolytica* and *Raoultella planticola*. The detection of such opportunistic pathogens indicates profound changes in the ecology of the wound process in the context of immunodeficiency states [7–9].

A detailed analysis of species composition is critically important for preventing the spread of the pathological process to deeper structures, including bone tissue.

In modern clinical practice, the Wagner classification is used to characterize the severity of ulcerative lesions and is the most widely applied system for assessing purulent-necrotic processes in the diabetic foot. This system enables diabetic ulcers to be classified into six levels based on wound depth. Level 0 indicates intact skin. Level 1 corresponds to a superficial ulcer. Level 2 denotes a deeper ulcer extending to tendons, bone, or joints. Level 3 represents a severe ulcer with an abscess or infected bone. Level 4 indicates the development of gangrene in the forefoot, while level 5 corresponds to gangrene involving the entire foot [6].

Prediction of the course of Diabetic Foot Syndrome and the development of individualized, comprehensive surgical treatment methods remain priority tasks, as a personalized approach can significantly reduce the number of major amputations. Assessment of the patient's condition using the Wagner classification allows for objective evaluation of lesion severity at the time of hospitalization. The combination of radical surgical debridement with pathogenetically justified correction of metabolic disorders is a key factor in improving the quality of life of such patients [10–12].

**The aim of the study:** to determine the relationship between the severity of purulent-necrotic lesions

in Diabetic Foot Syndrome in patients with Type 2 Diabetes Mellitus and the degree of microbial contamination according to the Wagner classification.

## Materials and Methods

Wound contents from 75 patients aged 45–75 years with Type 2 Diabetes Mellitus complicated by Diabetic Foot Syndrome were examined. The patients were treated in the surgical department of the Ternopil Municipal Emergency Hospital (MNE) during the period from January 2025 to February 2026. Among the patients included in the study, the state of subcompensation of carbohydrate metabolism predominated (93.5 %), whereas signs of decompensation were observed in 8.5 % of individuals. The clinical presentation at the time of hospitalization was characterized by pronounced purulent-necrotic lesions of various localizations in the foot region.

The patient cohort was formed based on an analysis of clinical forms and the duration of the underlying disease. Exclusion criteria included: the presence of type 1 diabetes mellitus, concomitant somatic pathology in the stage of decompensation, as well as cases of Diabetic Foot Syndrome without disruption of skin integrity. All patients included in the study had a complicated course of Diabetic Foot Syndrome with manifestations of purulent-necrotic lesions (abscesses, phlegmon, purulent tenosynovitis and arthritis, gangrene), which required urgent surgical treatment.

The severity and depth of purulent-necrotic soft tissue destruction were assessed by stratifying patients according to the Wagner classification [12].

Biological material (wound exudate, purulent content) was collected under aseptic conditions in compliance with current regulatory protocols. The obtained samples, placed in sterile tubes, were transported within 2 hours to the microbiological laboratory of the Department of Microbiology, Virology, and Immunology of I. Horbachevsky Ternopil National Medical University. Primary inoculation of the material was performed on a broad range of differential diagnostic and selective media. Incubation was carried out at +37 °C for 24–48 hours.

Comprehensive identification of the isolated pathogens included the use of bacterioscopic and bacteriological methods.

All studies were conducted in accordance with fundamental bioethical standards and the requirements of the Declaration of Helsinki, as confirmed by the conclusion of the Bioethics Committee of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine (Protocol No. 77 dated April 18, 2024).

## Results

The object of clinical and microbiological analysis was the wound content of 75 patients with Type 2 Diabetes Mellitus complicated by Diabetic Foot Syndrome. The age range of the examined cohort was 45–75 years.

The distribution of patients by sex and age characteristics demonstrated a predominance of males – 74.6 % (n=56) compared to females – 25.3 % (n=19). The mean age of patients was (64.3±4.7) years (Table 1).

A detailed analysis of the age structure revealed that the peak incidence occurred in the 61–70 age group. In this segment, the frequency of purulent-necrotic complications was the highest, accounting for 34.6 % among males and 13.3 % among females, which collectively defines this age category as the highest-risk group.

Comprehensive examination of patients was based on the use of clinical and instrumental methods, along with thorough analysis of complaints and medical history. The diagnostic program included verification of the type of diabetes mellitus and assessment of glycemic control (Table 2). Particular attention was paid to differentiation of the pathogenetic form of Diabetic Foot Syndrome, as well as detailed characterization of the localization, nature, and extent of the purulent-necrotic process.

According to the study results, the stage of subcompensation was identified in 61.3 % of male patients and in 17.3 % of female patients (Table 2). A

state of decompensation was observed in 21.3 % of all patients included in the study.

The severity and depth of purulent-necrotic tissue destruction in the foot were assessed in accordance with F. W. Wagner's classification criteria. According to the results of the analysis, grade I was diagnosed in only 2.7 % of cases (n=2), with the microbial flora comprising *S. aureus*, *R. planticola*, and *R. ornithinolytica*; Grade II was confirmed in 14.6 % of patients (n=11) with the presence of *E. faecalis* and *S. aureus*; Grade III was not observed in the study cohort (0 %); Grade IV was the most common and was recorded in 82.7 % of patients (n=62), where the greatest species diversity was observed: *S. aureus*, *S. haemolyticus*, *P. aeruginosa*, *C. amycolatum*, *S. agalactiae*, *P. fragi*, *P. taetrolens*, *E. faecalis*, *Rothia amara*, *K. pneumoniae*, *P. alimentarius* (Table 3, Fig.). The main types of surgical interventions included necrectomy (49 %), minor amputations (toe amputations and resections of metatarsophalangeal segments) – 25 %, as well as transmetatarsal amputations – 31 %. At the same time, in 64–75 % of cases, necrectomy was only the first stage of treatment, followed by the need for repeated surgical revisions or extension of the intervention volume, depending on the spread of the pathological process.

According to the results of the microbiological study, the leading role in the microbial profile of Diabetic Foot Syndrome belongs to Gram-positive microflora, particularly representatives of the genera

**Table 1.** Age distribution of patients with T2DM and DFU

| Sex            | Women |      | Men  |      |
|----------------|-------|------|------|------|
|                | n=19  | %    | n=56 | %    |
| Up to 50 years | 0     | 0    | 5    | 6.6  |
| 51–60          | 7     | 9.3  | 20   | 26.6 |
| 61–70          | 10    | 13.3 | 26   | 34.6 |
| Over 70 years  | 2     | 2.6  | 5    | 6.6  |

**Table 2.** Distribution of patients with T2DM complicated by DFU according to the grade of carbohydrate metabolism compensation

| Sex   | Grade of compensation |   |                          |      |                         |      |
|-------|-----------------------|---|--------------------------|------|-------------------------|------|
|       | Compensation<br><10.0 |   | Subcompensation<br><13.9 |      | Decompensation<br>>13.9 |      |
|       | n=0                   | % | n=59                     | %    | n=16                    | %    |
| Women | 0                     | 0 | 13                       | 17.3 | 6                       | 8.0  |
| Men   | 0                     | 0 | 46                       | 61.3 | 10                      | 13.3 |

**Table 3.** Distribution of patients with DFU according to the depth of ulcer defect spread, according to the Meggitt – Wagner classification

| Sex   | Grade |     |      |     |     |   |      |      |     |   |
|-------|-------|-----|------|-----|-----|---|------|------|-----|---|
|       | I     |     | II   |     | III |   | IV   |      | V   |   |
|       | n=2   | %   | n=11 | %   | n=0 | % | n=62 | %    | n=0 | % |
| Women | 1     | 2.7 | 4    | 5.3 | 0   | 0 | 11   | 14.7 | 0   | 0 |
| Men   | 0     | 0   | 7    | 9.3 | 0   | 0 | 51   | 68.0 | 0   | 0 |

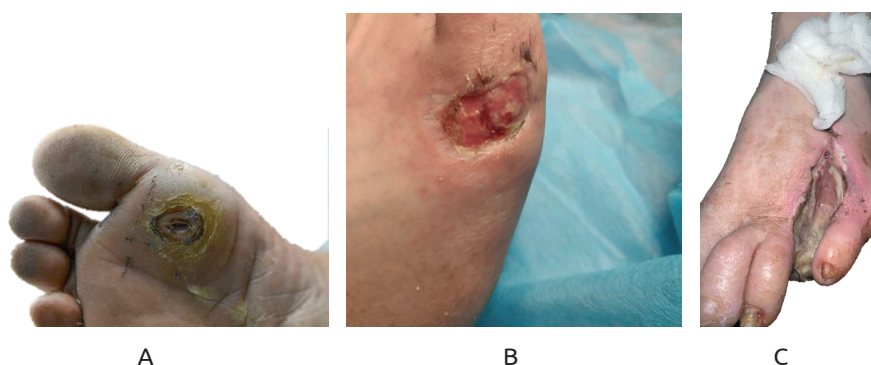


**Fig.** Grades of soft tissue involvement in patients with T2DM complicated by DFU according to the Wagner classification: A – Grade I, B – Grade II, C – Grade IV.

*Staphylococcus spp.* (31.0 %) and *Streptococcus spp.* (18.0 %).

A number of microorganisms, including *Micrococcus luteus*, *Streptococcus agalactiae*, *Acinetobacter lwoffii*, *Raoultella planticola*, and others, were identified with a frequency of less than 1.0 %.

Thus, the etiological structure of purulent-necrotic complications in Type 2 Diabetes Mellitus with Diabetic Foot Syndrome is predominantly formed by Gram-positive facultative anaerobic and aerobic cocci. At the same time, the leading role in the development of the pathological process belongs to *S. aureus*.

Transmetatarsal amputations were performed in cases of necrosis spreading to several rays of the foot or in total involvement of the forefoot. In this group of patients, polymicrobial associations predominated, involving *Klebsiella spp.* and *Pseudomonas aeruginosa*, which were characterized by a high level of antibiotic resistance. The presence of Gram-negative multidrug-resistant flora correlated with greater depth of tissue involvement, a more aggressive course of the infectious process, and the need for more extensive surgical intervention. Thus, a clear relationship is observed: Gram-negative multidrug-resistant microflora → more severe course → higher level of amputation.

## Discussion

A characteristic feature of Type 2 Diabetes Mellitus is the tendency to develop various complications, among which a leading place is occupied by Diabetic Foot Syndrome, venous trophic ulcers of the lower extremities, as well as neoplastic and postoperative wounds. One of the determining factors that slows reparative processes and impairs physiological wound healing is bacterial infection. According to scientific data, the clinical manifestation of infection in the course of Diabetic Foot Syndrome is observed in approximately 30 % of patients [13, 14].

Given the complexity of the disease course, particular attention is paid to international classifications that allow standardization of the approach to assessing the severity of the patient's condition.

The Wagner scale is widely used to evaluate the severity of lesions and is based on the analysis of a range of local signs: hyperemia, edema, pain syndrome, wound size and depth, as well as the risk of amputation in severe cases.

At Grade 0 the skin of the foot remains intact, deformities, dryness, or calluses may be present, but without signs of ulceration, indicating an increased risk of further lesions; Grade 1 is characterized by the presence of a superficial ulcer limited to the epidermis and dermis; Grade 2 – the ulcerative process extends deeper and affects subcutaneous tissue and soft tissues, often accompanied by the development of infection; Grade 3 – a deep ulcer is observed, which may be associated with the formation of abscesses, osteomyelitis, or septic arthritis; Grade 4 is associated with the development of localized gangrene (dry or wet), involving one part of the foot, for example a toe, with necrosis of all tissue layers in this area; Grade 5 is characterized by the presence of extensive gangrene affecting a significant part or even the entire foot, often requiring radical surgical intervention, including amputation [15].

The intensification of tissue hypoxia and impairment of microcirculation, in combination with existing ischemia, are consequences of the superimposition of bacterial infection in the wound process.

The microbiota of Diabetic Foot Syndrome is a dynamic structure functioning within a polymicrobial matrix of purulent-necrotic lesions. Complex interspecies interactions of microorganisms in such an environment determine the pathogenesis of chronicity and significantly inhibit regenerative processes. According to the analysis of the literature, the most common isolates identified in diabetic ulcers include *Staphylococcus aureus*, *Streptococcus spp.*, *Klebsiella spp.*, *Corynebacterium spp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis* [4, 5, 7].

The complex nature of the disease requires the use of modern and clinically validated classification systems, one of which is the PEDIS classification (Perfusion, Extent/size, Depth/tissue loss, Infection).

It encompasses not only the vascular component but also key parameters of the lesion, including the presence and nature of the infectious factor. In patients with Diabetes Mellitus, due to an impaired immune response, the infectious process develops rapidly, often with subtle clinical manifestations at early stages. All of the above significantly complicates timely diagnosis, allowing the infection to penetrate deep tissues, including muscles, fascia, and bone, causing osteomyelitis and leading to generalization of the process in the form of sepsis [16].

### Conclusions

1. The results of the clinical and microbiological analysis confirm that Diabetic Foot Syndrome in Type 2 Diabetes Mellitus is not only an infectious but primarily a surgical problem requiring early and radical intervention. The high frequency of Grade IV according to the Wagner classification (82.7 %) indicates the

predominance of patients with deep purulent-necrotic lesions, gangrene, and probable involvement of bone structures, necessitating organ-preserving or amputation procedures.

2. The presence of polymicrobial microflora involving *Klebsiella* spp. and *Pseudomonas aeruginosa* has been established; these are characterized by a high level of antibiotic resistance and directly influence the severity of the infectious process.

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### Conflict of interest

None.

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## Мікробіологічна характеристика виразкових уражень при цукровому діабеті 2 типу з урахуванням класифікаційної моделі Wagner

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**Анотація.** Відповідно до моніторингових досліджень, щорічно відбувається збільшення числа хворих на цукровий діабет (ЦД) 2 типу серед населення України в середньому на 5–7 % із подальшим розвитком синдрому діабетичної стопи (СДС), патогенез якого зумовлений масивною мікробною контамінацією. Комплексний підхід до діагностики та лікування даної патології, який включатиме як мікробіологічний аспект, так і використання різноманітних класифікаційних шкал, сприятиме стандартизації клінічної оцінки та полегшуватиме вибір лікувальної тактики. Метою дослідження було встановити залежність ступеня тяжкості гнійно-некротичних уражень при СДС у хворих на ЦД 2 типу від мікробної контамінації відповідно до класифікації Wagner. У дослідженні використано рановий вміст, одержаний від 75 пацієнтів із ЦД 2 типу, ускладнений СДС. Оцінку тяжкості деструкції м'яких тканин проводили шляхом стратифікації хворих із використанням класифікації Wagner. Мікробіологічний аналіз здійснювали на базі кафедри мікробіології, вірусології та імунології Тернопільського національного медичного університету імені І. Я. Горбачевського МОЗ України. Результати дослідження показали, що пік СДС був у віковій групі 61–70 років: 34,6 % – серед чоловіків та 13,3 % – серед жінок. Оцінка ступеня компенсації вуглеводного обміну показала переважання стадії субкомпенсації (78,6 %). Згідно з класифікацією Wagner, домінуючим (82,7 %) виявився IV ступінь, при якому верифікувалися штами *Klebsiella* spp. та *P. aeruginosa*. Таким чином, за класифікацією Wagner встановлено високу (82,7 %) частоту IV ступеня та переважання глибоких гнійно-некротичних уражень з наявністю антибіотикорезистентних штамів *Klebsiella* spp. та *P. aeruginosa*.

**Ключові слова:** цукровий діабет; синдром діабетичної стопи; мікроорганізми; класифікація Wagner; мікрофлора.