

УДК 616-008.9-021.6-02:616.12/.13]-092.9
<https://doi.org/10.20538/1682-0363-2022-3-166-180>

Pathogen-specific molecular imaging and molecular testing methods in the prognosis of the complicated course of diabetic foot syndrome, the risk of amputation, and patient survival

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ABSTRACT

The aim of this review was to provide extended information on current trends in the diagnosis of complicated diabetic foot syndrome (DFS), the most frequent and severe complication of diabetes mellitus, including high-tech medical imaging methods and instrumental and laboratory predictors of the complicated course and risk of amputation in DFS.

The article provides an analytical review of modern publications over the past 5 years on diagnosis and therapy. Pilot data on the use of high-tech medical imaging methods, assessment of skin microbiota and ulcers in DFS, molecular testing methods in terms of predicting the amputation risk and survival of patients with DFS, as well as the effectiveness of biosensing systems have been systematized, summarized, and subjected to analytical evaluation.

The review provides an expert assessment of the capabilities of pathogen-specific molecular imaging using modern positron emission tomography (PET), single-photon emission computed tomography (SPECT), and high-energy radionuclides in bacterial infection to understand its pathogenesis, minimize diagnostic problems, improve antimicrobial treatment, and address fundamental and applied aspects of DFS. Literature data on the assessment of foot perfusion in diabetic patients with varying degrees of limb ischemia by hybrid technologies (SPECT / CT and PET / CT) and new modalities of magnetic resonance imaging (MRI) are also systematized, which contributes to new understanding of the response to revascularization, surgical shunting, and stimulation of angiogenesis within ischemic tissue, as well as potentially to healing of foot ulcers.

The review is aimed at substantiating a multidisciplinary approach in DFS, selection, development, and implementation of innovative strategies for diagnostic modalities to identify diabetic foot pathologies, and choice of an adequate method for treating and monitoring the results of therapy in the context of personalized medicine.

Keywords: diabetes mellitus, diabetic foot syndrome, osteomyelitis, angiosome, perfusion, microbiota, molecular imaging

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The study was supported by the RFBR grant within the research project No. 20-115-50333.

For citation: Zorkaltsev M.A., Zavadovskaya V.D., Saprina T.V., Zamyshevskaya M.A., Udodov V.D., Shestakov A.V., Mikhailova A.A., Loyko Yu.N., Musina N.N. Pathogen-specific molecular imaging and molecular testing methods in the prognosis of the complicated course of diabetic foot syndrome, the risk of amputation, and patient survival. *Bulletin of Siberian Medicine*. 2022;21(3):166–180. <https://doi.org/10.20538/1682-0363-2022-3-166-180>.

Патоген-специфическая молекулярная визуализация и методы молекулярного тестирования в прогнозе осложненного течения синдрома диабетической стопы, риска ампутаций и выживаемости пациентов

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РЕЗЮМЕ

Цель настоящего обзора – расширение информации об актуальных направлениях в диагностике осложненного течения синдрома диабетической стопы (СДС), частого и тяжелого осложнения сахарного диабета, включая высокотехнологические методы медицинской визуализации и инструментально-лабораторные предикторы осложненного течения и риска ампутаций при СДС.

Представлен аналитический обзор современных публикаций за последние 5 лет по диагностическим и терапевтическим направлениям; систематизированы и обобщены, а также подвергнуты аналитической оценке пилотные данные, касающиеся использования высокотехнологических методов медицинской визуализации, оценки микробиоты кожи и язвенных дефектов при СДС, методов молекулярного тестирования с точки зрения прогноза риска ампутаций и выживаемости пациентов с СДС и эффективности применения систем биосенсирования.

Дана экспертная оценка возможностей патоген-специфической молекулярной визуализации с использованием современных технологий позитронно-эмиссионной томографии (ПЭТ) и однофотонной эмиссионной компьютерной томографии (ОФЭКТ) и высокоэнергетических радионуклидов при бактериальной инфекции для понимания ее патогенеза, минимизации диагностических проблем, улучшения антимикробного лечения и для решения фундаментальных и прикладных аспектов СДС. Систематизированы литературные данные об оценке перфузии стоп у больных сахарным диабетом с различной степенью ишемии конечностей методами гибридных технологий (ОФЭКТ/КТ и ПЭТ/КТ) и новых модальностей магнитно-резонансной томографии, что способствует новому пониманию ответной реакции на реваскуляризацию, хирургическое шунтирование и стимулирование ангиогенеза в пределах ишемизированной ткани, а также потенциально – и для заживления язвы стопы.

Статья направлена на обоснование мультидисциплинарного подхода при СДС, а также выбор, развитие и внедрение инновационных стратегий диагностических модальностей в установлении патологических процессов при СДС, выбор адекватного метода лечения и мониторингирования результатов терапии в рамках развития персонализированной медицины.

Ключевые слова: сахарный диабет, синдром диабетической стопы, остеомиелит, ангиосома, перфузия, микробиота, молекулярная визуализация

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с содержанием настоящей статьи.

Источник финансирования. Исследование выполнено при финансовой поддержке РФФИ в рамках научного проекта № 20-115-50333.

Для цитирования: Зоркальцев М.А., Завадовская В.Д., Саприна Т.В., Замышевская М.А., Удодов В.Д., Шестаков А.В., Михайлова А.А., Лойко Ю.Н., Мусина Н.Н. Патоген-специфическая молекулярная визуализация и методы молекулярного тестирования в прогнозе осложненного течения синдрома диабетической стопы, риска ампутаций и выживаемости пациентов. *Бюллетень сибирской медицины*. 2022;21(3):166–180. <https://doi.org/10.20538/1682-0363-2022-3-166-180>.

INTRODUCTION

The problem of diabetic foot syndrome (DFS), which is the most common and severe complication of diabetes mellitus (DM) due to high percentage of post-amputation deaths, remains critical and requires a multidisciplinary approach to monitor and predict the therapeutic response. The aim of this review was to provide extended information on current trends in the diagnosis of complicated DFS, including high-tech medical imaging methods and instrumental and laboratory predictors of the complicated course and risk of amputation in DFS.

The main manifestation of DFS is trophic ulcers or other purulent and destructive processes starting from the integumentary system. However, the cause and pathogenetic mechanisms of phenotypically similar DFS manifestations can be completely different. It complicates the choice of adequate therapeutic strategies (conservative, surgical, as well as their combinations) and the prognosis of this DM complication, increasing the incidence of poor outcomes and the number of amputations of lower extremities and post-amputation deaths.

The main pathogenetic mechanisms of DFS are the development of diabetic macroangiopathy, microangiopathy, neuropathy, in complicated forms – bacterial infection in the soft tissues and bones of the feet. The underlying pathology in a number of patients, which could contribute to the main mechanisms of DFS formation (joint damage, chronic venous insufficiency, radicular syndrome, etc.), complicates the choice of the correct treatment strategy. Under these conditions, it is extremely relevant to identify the leading pathogenetic mechanisms of the DFS formation, evaluate the contribution of the underlying pathology, and develop and validate new pathogenetically grounded radiological methods and methods of molecular diagnosis and biosensing.

MODERN DIAGNOSTIC RADIOLOGY METHODS IN DFS

Magnetic resonance imaging in diabetic foot syndrome

Most authors consider magnetic resonance imaging (MRI) as the gold standard in diagnostic radiology of complicated DFS [1–3]. Technical capabilities of MRI in recent years make it possible

to use this method in DFS both to visualize structural changes in the foot tissues and to assess the vascular bed using contrast-enhanced and non-contrast-enhanced MR angiography. An important advantage of MRI, according to a recent review by C. A. Ruiz-Bedoya et al. (2019), is recognition of bone marrow edema with precise anatomical localization at an early stage – 3–5 days after the onset of the infectious process [4].

Recent literature sources describe MR-semiotics of foot lesions in diabetes mellitus (DM) in detail. According to D. H. Massel et al. (2020), MRI has high sensitivity in the diagnosis of osteomyelitis (OM) of the foot due to typical changes in signaling characteristics and allows to accurately localize purulent complications and assess their prevalence and clinical risk of complications [5]. Recent publications indicate the possibility of using certain MR signs as predictors of purulent and destructive complications in DFS. Thus, bone marrow edema and an adjacent at this level subcutaneous adipose tissue ulcer with the depth of more than 50% are unambiguous predictors of the development of OM of the foot [6, 7].

Moreover, Y. H. Jang et al. (2020), based on the results of a multivariate analysis of MRI data, indicated a new pattern of DFS complication development: confluent pattern of decreased T1 marrow throughout the entire bone fragment, as well as “blotches” of unchanged bone marrow against the background of a “thin / reticular mesh” [7].

H. M. Kotecha et al. (2020) currently consider the possibility of using native MRI in emergency departments in patients with DFS as an abbreviated MRI protocol (only coronal T1-weighted and sagittal T2-weighted FMPIR images) lasting an average of 8 minutes [8]. A number of authors have substantiated MRI in patients with DFS for planning the scope of surgical interventions. The data obtained by M. Jbara et al. (2016) indicate a significant association of preoperative MRI with a decrease in post-operative mortality [9, 10]. The relevance of repeat MRI for assessing treatment outcomes in patients of reproductive age with DFS is reported by C. Lauri et al. (2018) [11].

However, D. Duryea et al. (2017) note that, despite high sensitivity and positive and negative predictive power of MRI in the diagnosis of purulent complications, the specificity of the method is not

so high due to the difficulties in differential diagnosis of aseptic and inflammatory bone marrow edema [5, 7]. Therefore, differential diagnosis of pathological changes in the feet remains a frequent clinical problem in DM patients [12].

Contrast-enhanced and non-contrast-enhanced MR angiography

Contrast-enhanced MR angiography (CE-MRA) is of great importance in assessing the anatomy and pathology of the foot vessels. The advantages of CE-MRA over digital subtraction angiography (DSA) in patients with DFS are visualization of a larger number of stenotic vessels and simultaneous clear visualization of inflammatory complications, such as OM, soft tissue abscesses, and fistulas [13]. However, CE-MRA is inferior to SA in assessing distally located vessels, as well as in critical ischemia cases. A number of publications indicate the priority of this technique before revascularization procedures in patients with ischemic or mixed forms of DFS and refusal to use X-ray techniques in foreign medical centers [12].

Preoperative CE-MRA assesses arterial patency, the presence of diffuse calcification in distal vessels and microaneurysms, the state of collateral blood flow, and targeted vessels for surgical bypass [14]. CE-MRA is useful for identifying active-phase Charcot foot, monitoring a response to treatment, and predicting the healing time of wound-related defects [1, 3]. The diagnostic efficiency parameters of CE-MRA exceed those of CT angiography (CTA) and duplex ultrasound scanning of the foot vessels in DFS [13].

MR-semiotics of complicated DFS has been sufficiently studied to date. Thus, M.A. Zamyshvskaya et al. (2016) presented MRI data on blood supply to the foot in case of intraosseous purulent inflammation, acute diabetic osteoarthropathy, and arteriovenous shunting. The authors described the distribution of the contrast agent with an increase in vascular permeability in inflammation sites [15].

The quantitative assessment of dynamic contrast-enhanced MRI is described in sufficient detail [13, 16]. D. Liao et al. (2018), using the extended linear Tofts model, evaluated three quantitative parameters of CE-MRA: the transfer constant – K_{trans} , the contrast rate index – K_{ep} , and the volume frac-

tion of the extravascular extracellular space – V_e [16]. These parameters demonstrated statistical significance in the differential diagnosis of Charcot foot and OM in DFS, however, the limited number of patients and the peculiarities of post-processing do not yet confirm the possibility of wide use of the quantitative assessment of MR angiography data in clinical practice.

Technological progress made it possible to conduct not only contrast-enhanced, but also non-contrast-enhanced studies of the peripheral vessels in the lower extremities, which are based on either an increase or decrease in the blood flow signal. N. Zhang et al. (2016) in their publication concluded that non-contrast-enhanced MRA can be used as a safe and reliable screening tool for assessing the state of the foot arteries in patients with DM [17].

Quiescent-interval single-shot MR angiography (QISS MRA) is a two-dimensional, balanced, steady-state, free precession synchronized with ECG, which has several advantages and demonstrates high accuracy compared with CTA and cMRA in the diagnosis of critical lower limb ischemia in DFS. A variant of this modality, QISS MRA with arterial spin labeling (ASL), is a related technique that has the potential to visualize the foot vessels due to theoretically improved background suppression.

A. Lam et al. (2020) described in detail the use of QISS MRA and QISS ASL-MRA in patients with critical ischemia due to DFS, which was not done in earlier studies [18]. The authors describe these non-contrast-enhanced MRI techniques as fast, relatively simple, and at the same time highly effective imaging methods that are insensitive to patient movements, pulse wave, and blood flow characteristics, as well as applicable in the presence of severe diabetic nephropathy with a decrease in the estimated glomerular filtration rate (eGFR). At the same time, QISS MRA is considered as a method of targeted visualization for performing shunting and revascularization of the foot vessels. The use of more powerful MRI machines (3T) and improvement of a number of technical aspects can improve this non-contrast-enhanced MRI technique and contribute to its introduction in clinical practice.

Diffusion-weighted MRI in DFS

Foreign publications on the use of diffusion-weighted MRI to detect the inflammatory process in DFS appeared in 2017–2020 [12, 19, 20]. The publication by A.A. K. Abdel Razek and S. Samir (2017) indicates that one of the advantages of this technique is the ability to quantify the signal from altered foot tissues by calculating the diffusion coefficient (DC) [19]. The DC values in the affected bone are significantly higher in acute diabetic neuroarthropathy compared with those in OM. Thus, diabetic osteoarthropathy is associated with bone marrow edema with a relatively smaller inflammatory cell number and higher DC values, while OM is associated with the presence of microorganisms, as well as inflammatory and dead cells, hence, with subsequent limited diffusion and lower DC values. However, diffusion-weighted MRI does not completely exclude the combination of osteoarthropathy and OM, especially at the early stage of inflammation and with the subacute course of neuroarthropathy, which reduces the diagnostic efficiency of the technique [19].

The use of 3.0T MRI systems with new technical characteristics aimed at suppressing perfusion effects can improve the accuracy of DC calculations, contributing to the improvement of the qualitative and quantitative characteristics of diffusion-weighted images in DFS [12]. At the same time, it is too early to raise the issue of using diffusion-weighted MRI in clinical practice due to a lack of standardization for DC calculation, subjectivity of interpretation of the obtained values, and technical capabilities of MRI machines, which requires a larger study or meta-analysis of the use of diffusion-weighted MRI in DFS.

Radionuclide diagnosis in DFS

Scintigraphy with ^{99m}Tc -HMPAO- and ^{111}In -oxime-labeled leukocytes is regarded as the gold standard in radionuclide diagnosis of OM in DFS [1, 21]. In 2018, European Association of Nuclear Medicine (EANM) in order to standardize the procedure for labeling leukocytes developed research protocols and criteria for interpreting the method results. These criteria include comparative characteristics of various degrees of radiopharmaceutical hyperfixation at control points (after 1, 3, and 20 hours) depending on sterile and non-sterile inflammation [22].

Concomitant Charcot osteoarthropathy, leading to radiopharmaceutical hyperfixation due to increased hematopoietic activity in the bone marrow, secondary to chronic inflammation, contributes to lower specificity of the method. To overcome this limitation, it is proposed to perform additional bone marrow scintigraphy using nanocolloids [11]. In case of using a dual technique, two diagnostic criteria for OM in Charcot arthropathy are described: capture of labeled leukocytes without corresponding activity on bone marrow scintigraphy images and incongruent spatial distribution of two radiopharmaceuticals [11, 23].

The main reasons for the decrease in the specificity of this technique are low anatomical spatial resolution, hyperfixation of labeled leukocytes at the site of sterile inflammation in the Charcot foot, and leukopenia [24]. Combined use of labeled leukocyte scintigraphy with a modality that is highly informative in terms of imaging anatomical structures can increase the specificity of this method.

The effect of long-term antibiotic treatment on the sensitivity of labeled leukocyte scintigraphy is still a matter of debate. Judging by preliminary data, the use of radioactively labeled leukocytes is allowed 2 weeks after the end of therapy, even in the presence of false negative results [1, 22].

As an alternative to labeled leukocytes, monoclonal antibodies (MoAbs) or antibody fragments (Fabs) against specific antigens expressed by activated granulocytes can be proposed. However, the role of MoAbs and Fabs in assessing complicated DFS has not been widely studied, there are no standardized protocols for collecting and interpreting research results [11].

There are some recent studies developing highly specific biomolecules and new agents, since molecular imaging of bacterial infections may provide a unique opportunity to monitor treatment of patients with DFS complicated by infections. Currently, in this category of patients, according to A.O. Ankrah et al. (2018), the results of using a number of isotope indicators, such as ^{18}F -FDS, ^{99m}Tc -UBI 29-41, and ^{68}Ga -NOTA-UBI, are evaluated. Using these isotope indicators does not require manipulations with blood and is able to differentiate between inflammatory and infectious processes with high specificity [25]. However, the sensitivity of these agents requires further evaluation and confirmation

in larger clinical trials, especially in cases of chronic infections with low bacterial load [4].

Scintigraphy with labeled antibiotics, such as ^{99m}Tc -labeled ciprofloxacin, can identify the infectious process and differentiate between sterile and non-sterile inflammation, but this radiopharmaceutical has lower diagnostic efficiency than radiopharmaceuticals with labeled leukocytes, which is probably due to a non-specific accumulation mechanism. In 2019, a group of researchers led by N. Ahmed published the results of ^{99m}Tc -labeling of ceftizoxime, a third-generation cephalosporin with a broad spectrum of antibiotic activity compared with ciprofloxacin [24].

J. Vouillarmet et al. (2017) report the use of single-photon emission computed tomography (SPECT) / CT with labeled leukocytes to predict remission after a 6- or 12-week course of antibiotic therapy in the case of non-surgical treatment of foot OM in DM [26]. However, W. J. Jeffcoate, basing on a number of publications (2016–2017) on comparing the effectiveness of clinical and instrumental monitoring of patients with complicated DFS, concludes that the use of this hybrid technique can be overestimated, and the method itself does not have a great impact on daily clinical practice [27].

According to R. Ahluwalia et al. (2020), SPECT / CT is a useful method of functional and structural imaging for both foot OM and Charcot foot [28]. At the same time, a broader and more prospective approach to the study of SPECT / CT as a method for identifying predictors of Charcot foot formation is required, taking into account the advantages of this method over MRI in assessing structural changes, as well as a method used in patients for whom MRI is contraindicated [1].

Positron emission tomography in DFS

^{18}F -fluorodeoxyglucose (^{18}F -FDG) positron emission tomography (PET) provides non-invasive 3D imaging with higher spatiotemporal resolution and sensitivity compared with SPECT and MRI, and moderate hyperglycemia does not affect the diagnostic performance of ^{18}F -FDG [2, 29]. The disadvantage of the method is low specificity. In addition, glucose uptake may remain impaired for 3–4 months after surgery or injury [4].

C. Ruiz-Bedoya et al. (2019) note that molecular imaging of bacteria at the site of infection facili-

tates selection of appropriate empiric antimicrobial therapy [4]. A drug like ^{18}F -fluorodeoxysorbitol (^{18}F -FDS), selectively targeting the Enterobacteriaceae family of bacteria, can be used in combination with a broad-spectrum imaging marker (e.g., ^{11}C -para-aminobenzoic acid) for PET indication of infection and differentiation between Gram-positive and Gram-negative bacteria.

Taking into account the advantages of PET over SPECT imaging, it is recommended to study and develop PET equivalents of indicators that are potential for visualizing infectious agents. The use of radiometals, such as ^{68}Ga , offers more labeling possibilities, especially for peptides. Radionuclides with longer half-lives, such as ^{64}Cu and ^{89}Zr , can also be a good alternative for labeling peptides currently labeled with ^{68}Ga . These relatively longer-acting radionuclides will provide delayed imaging, which is a requirement for infection indication, including those in DFS. Radioimmunotherapy with antibiotics using nanoparticles labeled with new radionuclides for drug delivery can shorten the duration of antibiotic therapy and affect resistant microorganisms [25].

According to C. Lauri et al. (2020), ^{18}F -FDG PET / CT is an alternative to scintigraphy, however, to date, there is no clear definition of criteria and standardization of this hybrid technique [1]. Thus, some authors note lower sensitivity of the hybrid technology in diagnosing complicated DFS compared with MRI, while the specificity and accuracy of the PET / CT method are significantly higher than those of MRI [12].

A semi-quantitative analysis of the maximum standardized absorbance value (SUVmax) was developed when performing ^{18}F -FDG PET / CT. C. Lauri et al. (2020) report significantly higher SUVmax values in cases of OM in DFS compared with the same parameter in Charcot foot and uncomplicated DFS, which indicates the significance of the SUVmax parameter for the differential diagnosis of these pathological processes [23]. Although A.I.G. Diez et al. (2020) also concluded that the SUVmax parameter can be useful for differentiating pathological processes in the foot in DM, some authors did not find statistically significant differences when comparing this parameter in patients with Charcot foot and OM [12].

Publications on the possibilities of PET / MRI in assessing the state of the bone marrow and monitoring the course of the disease in DFS patients with the subsequent prospect of differentiating damage to soft tissue and bone structures [12] reflect the development of hybrid techniques. A.W.J.M. Glaudemans et al. (2019) believe that PET / MRI, as opposed to PET / CT, will increase the accuracy of diagnosing foot infections by improving the differentiation of OM itself and soft tissue infections [30]. The potential possibility of obtaining hybrid SPECT / MRI images using workstation software is studied [31].

A few foreign articles describe various approaches to the quantitative / semi-quantitative assessment of visual data in patients with DFS and the creation of clinical diagnostic scales. A variant of the MR scoring system for patients with Charcot foot was presented by L. Meacock et al. in 2017. The presented semi-quantitative scale was based on such MRI symptoms as bone marrow edema and the presence of the affected bone fracture [32].

V.D. Udodov et al. patented and described in the dissertation thesis (2018) a scoring system for assessing combined SPECT / MRI in patients with DFS and suspected OM. This system is currently one of a kind for diagnostic practice. This scoring system includes a number of MR symptoms of inflammatory processes in DFS and two scintigraphic symptoms based on the results of labeled leukocyte scintigraphy. Based on the results of the ROC analysis, the threshold value for OM in DFS was obtained with a total score of existing visual symptoms of more than 12. This quantitative criterion is highly specific and sensitive and may become promising in the clinical diagnostic evaluation of the foot damage in DM patients.

ASSESSMENT OF TISSUE PERFUSION IN DFS

A significant problem in DM patients is impaired tissue perfusion and, as a result, endothelial dysfunction, capillary microangiopathy, and critical ischemia of the lower extremities [4, 33]. Targeted treatment of ischemic non-healing foot ulcers requires angiosome-directed revascularization, which could lead to a significantly higher rate of wound healing and reduce a risk of large limb amputations in patients with peripheral arterial disease (PAD) [34].

J.L.Alvelo et al. in 2018 described the use of ^{99m}Tc-tetrofosmin for perfusion imaging by SPECT / CT. The method demonstrated the possibility of a qualitative and quantitative assessment of foot microcirculation at rest with the ability to detect perfusion defects in areas containing non-healing foot ulcers in patients with critical lower limb ischemia and PAD [33]. It should be noted that ^{99m}Tc-sestamibi, used in cardiology, also showed high informative value and certain advantages at the preclinical and clinical stages in the diagnosis of PAD and was considered as an alternative to ^{99m}Tc-tetrofosmin in future investigations [33].

Regardless of previous scientists, a research group led by T. Chou in 2020 published their own data on the use of SPECT / CT for the quantitative assessment of angiosome perfusion as an additional method in relation to X-ray angiography and measurement of ankle – brachial index (ABI) and toe – brachial index in patients with DM and critical limb ischemia with planned lower limb revascularization [35].

Non-contrast-enhanced MRA is a highly effective method for the absolute assessment of foot perfusion in patients with DM. The results presented by H. Chen in 2018 suggest that the parameters of microvascular arterial injury in MRI will allow for a better understanding of the pathophysiology of ongoing changes in the tissues [36].

J. Zheng et al. (2016) published data on the possibilities of constructing non-contrast MRI perfusion angiosome foot maps in healthy individuals and patients with DM [37]. Foot perfusion measurements were taken during exercise. As a result, according to non-contrast MRA data, regional differences in the perfusion of the foot muscles in the isolated angiosomes were clearly visualized. In DM patients, perfusion during exercise was statistically significantly lower than in healthy volunteers.

In 2019, the same group of researchers led by M. Edalati published an extended version of the foot MR perfusion assessment using arterial spin labeling (ASL) [38]. The authors found that the MR perfusion reserve of the foot in DM patients was significantly lower than in patients without DM. The second important point was a statistically significant decrease in MR perfusion of the muscles in the per ulcer anatomical region both at rest (reserve) and during exercise compared with areas of the foot

located at a distance from the diabetic ulcer. Thus, the authors confirm and describe the angiosome theory of blood supply to the feet and its impact on the DFS course, however, due to the small sample size, additional experimental and clinical studies are required to confirm the results obtained. The main focus of this study, except for the angiosome theory, is the fact that the perfusion reserve of muscles around healed ulcers in DFS was significantly higher than that in long-term non-healing foot ulcers. This fact justifies the prospect of further study of MR perfusion reserves of skeletal muscles as predictors of foot wound healing in DFS [38].

Despite the initial success of angiosome-directed revascularization, up to 54% of foot ulcers cannot be unambiguously attributed to a specific angiosome due to dual blood supply. Recent studies of foot tissue in the near-infrared spectrum have made it possible to consider the image of oxygen saturation of the foot tissue (StO₂) as a modified angiosome theory. This new “angiosome” turned out to be better than the traditional angiosome model for detecting ischemic skin lesions associated with foot ulcers. Studies of tissue oxygenation in DM patients have continued in the evaluation of skeletal muscle oximetry [37].

An MRI-based assessment of microcirculation was carried out to measure the skeletal muscle oxygen extraction fraction (SMOEF) of the foot in DFS as well as to compare two angiosome models – classical and oximetric ones. According to the results, an absolute mean value of SMOEF at rest in DFS patients was higher than in healthy people. This difference may be due to reduced tissue perfusion in DFS patients, and, therefore, tissue oxygen demand must be met by increasing oxygen extraction even at rest. A clinically important point is the increase in SMOEF in healthy volunteers during the transition from rest to physical activity, rather than in patients with DFS when assessing similar angiosomes. It is worth noting that the oximetric angiosome model of the foot allows to directly determine the areas with low or high oxygen content delivered through one or more foot arteries. This distribution of angiosomes may be more appropriate for DM patients, since local microcirculation disruption is a frequent and clinically important aspect for patients with DM, even without peripheral arterial

occlusive disease, reflected by the classical angiosome theory [38].

Blood oxygenation level-dependent non-invasive functional MRI (BOLD-MRI) is a method for assessing dynamic changes in oxygenation of skeletal muscles, which reflects changes in the volumetric blood flow, especially in the microvasculature. The ability of the method to detect vascular anomalies of the foot may be especially valuable in patients with macro- and microvascular diseases [39].

Despite the widespread development of contrast-enhanced and the introduction of non-contrast-enhanced techniques for blood flow MR examination in DFS, most scientific publications are still limited to determining vascular patency. Other aspects of the blood flow are studied to a limited extent, and the data obtained are not enough to formulate reliable conclusions. Therefore, further exploring MRA possibilities, particularly, MR perfusion, in DFS is needed.

OTHER NON-INVASIVE DIAGNOSTIC METHODS AND PREDICTORS OF LOWER LIMB AMPUTATION IN PATIENTS WITH DFS

The relative simplicity and availability of radiation-free non-invasive methods for diagnosing DFS and predicting its course can provide the possibility of their use by a doctor directly during a patient consultation, as well as by DM patients themselves for daily monitoring and control of the foot state. The monitoring of blood pressure in the lower extremities, plantar temperature and pressure, and gait changes are among these methods. Complex biosensor systems designed to assess and / or monitor the presence of various markers of a poor disease prognosis in DFS, in particular, metalloproteinases and their tissue inhibitors, should also be mentioned. In addition to the above, a promising area of study is the assessment of the wound microbiome and microflora in DFS in order to diagnose and select a patient management strategy.

Monitoring of blood pressure and blood flow in the lower extremities

Evaluation of such physiological parameters as blood pressure and blood flow in the lower extremities showed high clinical significance in assessing the condition of patients with DFS. In a systematic review, R.O. Forsythe et al. (2020) noted the effec-

tiveness of 6 non-invasive clinical tests in predicting wound healing or a risk of amputation in DFS. The researchers identified 4 signs that showed the greatest accuracy in assessing the degree of a decrease in lower extremity perfusion, which in turn is an important indicator of a high risk of amputation: ankle pressure < 50 mm Hg, ABI < 0.5 , blood pressure in the toes < 30 mm Hg, as well as transcutaneous oxygen tension < 25 mm Hg.

On the other hand, in the same study, favorable signs indicating a higher likelihood of wound healing in DFS were also identified. Good outcome signs included skin perfusion pressure ≥ 40 mmHg, toe blood pressure ≥ 30 mmHg, and transcutaneous oxygen tension ≥ 25 mmHg. It is promising to use the results of these non-invasive tests in combination with other clinical predictors to select a strategy for further patient management – a variant of conservative treatment, a detailed assessment of perfusion disorders with possible subsequent revascularization or surgical treatment [40].

Monitoring of plantar foot temperatures

Monitoring of plantar foot temperature has shown itself to be a promising method for indirectly assessing the state of the blood flow in the lower extremities in order to early identify focal lesions of the feet. It was established that an increase in plantar temperature occurs as a result of repeated loading on the foot and inflammatory changes, in particular, enzymatic autolysis [41].

J. Golledge et al. in their research (2020) showed that monitoring the plantar foot temperature with a portable infrared thermometer on a daily basis is effective in preventing new or recurrent ulcers [42]. The parameter of temperature differences between symmetrical areas of opposite feet (2.2°C) turned out to be the most effective, as opposed to absolute temperature values and temperature comparison between areas of the same foot. However, despite the proven effectiveness, this method did not receive wide application value. The reasons for this are not entirely clear, but are likely to be explained by the difficulties for daily use and, as a result, low adherence of patients to this remote-control method. To overcome this barrier, innovative and ergonomic models are offered: floor mats for portable thermography, socks and insoles with built-in infrared sensors, portable infrared cameras for mobile phones

with the ability to continuously monitor changes in the parameter and send data to the attending physician [42,43].

In terms of compliance issues, the focus of researchers is increasingly shifting to complex multifunctional devices. In particular, temperature sensors that can be built into orthopedic shoes for diabetic feet are being developed. These sensors would make it possible to measure both the quality of foot unloading and the regularity of its wearing [44].

Thus, the greatest attention is paid to the development of complex sensors with the possibility of remote monitoring of parameters. This is due to increased attention both to telemedicine issues (taking into account the current epidemiological situation) and to issues of patient adherence to modern methods of diagnosis and treatment.

Plantar pressure measurement

Pressure, pressure gradients, shear stress, and peak plantar pressure are a group of mechanical loading parameters that contribute significantly to the formation of ulcers. The key point in the management of patients with DFS is the use of unloading devices and specialized shoes, made individually in accordance with the foot characteristics of each patient. In particular, one of the most important targets is reduction of peak plantar pressure to less than 25%, which makes a significant contribution to wound prevention in DFS [43, 44].

Plantar pressure monitoring is performed using pressure plates or insoles with built-in sensors in medical centers. However, some of the existing devices, such as Pedar® (*Novel, Munich, Germany*), F-Scan™ (*Tekscan Inc., USA*), and SurroSense Rx (*Orpyx Medical Technologies, Canada*), track the effectiveness of specialized orthopedic footwear in reducing peak plantar pressure in the outpatient setting [45].

A number of prototypes are able to warn the user about a prolonged increase in pressure (more than 35–50 mm Hg for more than 15 minutes) in a certain area of the foot using an audio signal, as well as to assess the degree of subsequent unloading of the foot [46]. Today, these systems are used primarily for research purposes, but are not integrated into clinical practice. Long-term monitoring of plantar pressure with the ability to provide feedback when “alarm

levels” occur is a promising avenue and certainly requires further study.

Monitoring gait changes

It is known that patients with diabetic polyneuropathy are characterized by impaired gait, which also contributes to changes in plantar pressure and to an increased risk of ulcers [47]. The greatest problem is the objectification of gait changes, since the patient himself can sometimes subjectively miss even pronounced disturbances in motor activity. In this regard, removable devices for remote monitoring of the step pattern have already been developed and tested [48]. At the next stage, these data can be used to develop training programs using artificial intelligence systems to correct gait disorders. For example, Samsung has created Gait-Enhancing Mechatronic System (GEMS) to redistribute foot pressure and reduce the risk of ulcers [49].

Biosensor systems

Wound bed assessment today relies mostly on subjective interpretation without the use of objective instruments. The use of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) as biomarkers in DFS is increasingly discussed by researchers.

To date, there is no suitable device that uses an objective quantitative index *in situ* to determine the severity of chronic ulcers, as well as a need for surgical treatment (in the outpatient setting, delayed surgical treatment often leads to aggravation of wounds). MMP-2 and -9 are considered as markers of active inflammation. Their expression increases during wound process exacerbation.

S. Kang et al. (2019) describe a hydrogel biosensor device for detecting the expression of MMPs in wound exudate, which allows to assess the severity of the ulcer and timely provide a surgical intervention. The mechanism of this biosensor model operation is as follows: upon immobilization of a fluorescent peptide cleaved by a specific metalloproteinase, its amount can be measured by an increase in the fluorescence of the hydrogel substrate [50].

A group of scientists from Bangkok in 2018 described changes in MMP-1, MMP-9, and TIMP-1 levels by ELISA during healing of diabetic ulcers. The authors concluded that the level of MMP-9,

MMP-1, and TIMP-1 could be used as a potential screening test during the patient's first visit. The researchers propose a scoring system which allows to divide patients into 2 prognostic groups at the clinical stage: ‘good healer’ and ‘poor healer’. This scoring can help to correctly determine a further treatment strategy. Moreover, it is expected that highly selective dressings with components that reduce the expression of the above inflammatory markers will be effective for diabetic ulcers that are difficult to treat with standard treatment methods [51].

Another promising technology is the multidimensional MS / MS protein identification system in tissue samples. Using this technology, the protein composition of foot exudates in DFS patients was analyzed. The study showed the overexpression of both metalloproteinases (MMP-1, MMP-2, and MMP-8) and proteins with antiangiogenic properties, such as collagens CO15A1 and CO18A1 [52].

J. Jones et al. in 2019 noted excessive activation of MMP-8 and MMP-9 in diabetic wounds in mice and humans, with MMP-9 being associated with longer healing of diabetic wounds, while MMP-8 expression was, on the contrary, a favorable prognostic factor for the disease [53]. Speaking of other biomolecules that play a key role in the healing of chronic ulcers, one cannot but mention growth factors (GF), such as EGF, bFGF, VEGF, PDGF, and IGF. It is known that the level of the above GFs in the wound is significantly reduced in DFS, which therapeutically justifies their use as topical drugs. The limiting factor in this case is low stability of the biomolecules *in vivo* due to degradation under the influence of tissue proteases in the wound bed. A promising direction is the development of sustained release drug delivery systems: solid lipid nanoparticles, nanostructured lipid carriers, polymeric microspheres and nanospheres, hydrogels, and nanofibrous scaffolds. Many studies in recent decades have confirmed their high therapeutic efficacy [54].

Microbiome

The microbiome is a collection of bacteria, viruses, unicellular eukaryotes, and other microorganisms coexisting with the host organism. Their presence creates a complex system of physiologically and metabolically interconnected processes

that affect a human life. The study of ulcer microbiome in DFS is of great importance. Identification of various bacterial strains in DFS plays an essential role in the prognosis of the disease. Ulcers can be colonized by various aerobic and anaerobic bacteria depending on various factors.

In superficial ulcers, the microbiome mainly consists of Gram-positive cocci, including *Staphylococcus aureus*, *Streptococcus pyogenes*, beta-hemolytic Streptococci, or coagulase-negative Staphylococci. Patients with deeper ulcers may be infected with Enterococci, *Pseudomonas*, or anaerobic bacteria [55].

One of the modern methods for studying the bacterial diversity of tissue samples is the method of culture analysis. This method involves the use of large-scale cultivation conditions for bacterial strains, followed by detailed identification of colonies using matrix-assisted laser desorption / ionization (MALDI) or 16S ribosomal RNA gene PCR. The method, according to the authors, makes it possible to identify all bacterial strains in the sample, including the most minimal ones, as a result, the entire microbiome of skin lesions in DFS was defined and described in detail. The authors found extremely high bacterial diversity, namely 53 species of various bacteria in the diabetic foot wound. A detailed analysis of bacterial diversity in each specific clinical case will allow for a more thorough approach to the selection of antibiotic therapy, however, this area requires further study.

Many studies demonstrate that the predominant bacterial strain in diabetic foot wound tissue is *Staphylococcus aureus*. It has also been found to be associated with a worse prognosis in diabetic foot infections, while the presence of *E. faecalis* in the wound significantly correlates with better wound healing. A number of factors have been found to in-

fluence the dominant bacterial strain establishment, such as demographic characteristics, personal hygiene, severity of the lesion, glycemic control, and current or previous antibiotic treatment. In addition, the laboratory research method used to identify bacteria also has a high impact [56].

M. Malone et al. in 2017 conducted a study using DNA sequencing to analyze the microbiota of the wound tissue in DFS. The study included groups of patients who received targeted antimicrobial therapy according to the results of a standard culture method. According to the results, no significant differences were found in the composition of the microbiome in patients with successful therapy and those who had no effect from the treatment, which calls into question the clinical significance of the standard culture method for determining bacterial wound strains in DFS [57].

In another research, J.U. Park et al. in 2019 used a DNA sequencing method to compare the microbiota of normal skin and ulcer in DFS. An important finding was the fact that the overall diversity of the bacterial flora was significantly poorer in diabetic foot wound tissue. It is assumed that the dominant development of opportunistic flora and local inflammation are mutually potentiating factors that lead to unfavorable clinical outcome. Moreover, it is noted that chronic inflammation in ulcers is a background for anaerobic flora, especially in the deep layers of the wound, which in turn causes an unfavorable clinical outcome. The predominant types of bacteria in the intact skin were Actinobacteria, Staphylococci, Corynebacteria, and Propionibacteria, while in ulcers, Anaerobes, Bacteroids, Enterococci, and Pseudomonads were found [58]. Summary data on the composition of the bacterial flora in normal skin tissue and tissue of diabetic foot ulcers are given in the Table [58].

Table

The microbiome of diabetic ulcers			
Parameter	Gram-positive bacteria	Gram-negative bacteria	Anaerobes
Predominant strains in diabetic foot ulcers	<i>Staphylococcus aureus</i> (MSSA and MRSA). <i>Streptococcus pyogenes</i> (beta hemolytic)	<i>Pseudomonas aeruginosa</i> . <i>Streptococcus pyogenes</i> (beta hemolytic). <i>Proteus</i> (different species)	<i>Peptostreptococcus</i> , <i>Bacteroides</i> , <i>Prevotella</i> , <i>Clostridium</i>
Localization of bacteria in the ulcer	Superficial layers of the wound	Superficial layers of the wound	Deep layers of the wound
Frequency of detection in DM	Non-predominant type of bacteria	Predominant type of bacteria	Present

Note: MSSA – methicillin-sensitive *Staphylococcus aureus*; MRSA – methicillin-resistant *Staphylococcus aureus*.

CONCLUSION

DFS and its complications are a common clinical problem. Delaying an accurate diagnosis can lead to an increase in patient complications, including amputation. The key questions regarding the diagnosis of foot infection, its location and spread, the type of pathogen, and the response to treatment are still not fully resolved, as accurate identification and differentiation of different types of DFS continues to be a challenge for clinical practitioners. Systematized in this article, special methods for providing valuable information about DFS and its complications will contribute to a better understanding of this disease. Multimodal imaging and a multidisciplinary clinical diagnostic approach, according to the authors, are mandatory in order to plan the most appropriate therapeutic strategy for an individual patient with DFS.

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Authors contribution

Zorkaltsev M.A. – conception and design. Zavadovskaya V.D., Saprina T.V. – editing of the manuscript and expert assessment. Zamyshvskaya M.A. – drafting and editing of the sections “Magnetic resonance diagnosis in DFS” and “Methods for assessing tissue perfusion in DFS”. Udodov V.D. – drafting and editing of the section “Radionuclide diagnosis in DFS”. Shestakov A.V. – drafting and editing of the section “Other non-invasive diagnostic methods and predictors of lower limb amputation in patients with DFS”. Mikhailova A.A. – drafting and editing of the section “Other non-invasive diagnostic methods and predictors of lower limb amputation in patients with DFS”. Loyko Yu.N. – drafting of the section “Methods for assessing tissue perfusion in DFS”. Musina N.N. – primary screening of the data, analysis and interpretation of the data. All authors approved the final version of the article before publication, agreed to be responsible for all aspects of the work, implying the proper study and resolution of issues related to the accuracy or integrity of any part of the work.

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Received 06.08.2021;
approved after peer review 23.09.2021;
accepted 05.10.2021