BMJ Open

Multifactorial strategies for the prevention of the risks of ulceration in patients affected by diabetic foot: a no-profit, multicentre, clinical trial protocol

Alberto Leardini ,[1] Uberto Pagotto,[2] Luca Dalla Paola,[3] Martina Piccinni Leopardi,[4] Claudio Belvedere,[1] Maurizio Ortolani,[1] Daniela Platano,[5,6] Paolo Caravaggi,[1] Giulia Rogati,[1] Giulio Sacchetti,[1] Michele S Grimaldi,[2] Giulia Casadei,[2] Valentina Lopreiato,[2] Elena Tremoli,[7] Francesco Maffessanti,[7] Elena Tenti,[7] Diego Sangiorgi,[7] Letizia Ferroni,[7] Anna Maria Malagoni,[7] Fabrizio Veglia,[7] Lisa Berti[5,6]

To cite: Leardini A, Pagotto U, Dalla Paola L, *et al*. Multifactorial strategies for the prevention of the risks of ulceration in patients affected by diabetic foot: a no-profit, multicentre, clinical trial protocol. *BMJ Open* 2026;**16**:e112163. doi:10.1136/bmjopen-2025-112163

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2025-112163).

Received 09 October 2025
Accepted 28 May 2026

For numbered affiliations see end of article.

Correspondence to
Dr Alberto Leardini;
LEARDINI@IOR.IT

ABSTRACT

Introduction Diabetes mellitus is a global epidemic affecting hundreds of millions of people worldwide. The diabetic foot is among the most serious complications, and it is estimated that between 15 and 25% of patients with diabetes mellitus develop ulcers during their lifetime. The costs for the treatments of foot ulcerations are enormous, and thus prevention and early personalised treatments are a priority. Many traditional clinical and metabolic analyses have been performed, but these should now be supported by modern specific multi-instrumental measurements.

Methods and analysis This is a no-profit, multicentre national study aimed at the prevention of complications in the diabetic foot. It is an interventional clinical trial, without medications or medical devices, involving two populations of patients with diabetes mellitus type-2. A first population has no previous history of foot ulceration but has a moderate ulcerative risk (International Working Group on the Diabetic Foot (IWGDF) grade 2); this will receive state-of-the-art clinical assessments and metabolic analyses in one centre, together with thorough biomechanical and functional analyses in a second centre and advanced biological and biochemical analyses in a third centre. A second population is affected by diabetic foot, had foot ulcers (IWGDF grade 3) but these must have been resolved for at least 6 months; in the centre in which they are recruited, they will receive state-of-the-art clinical assessments and metabolic analyses, together with advanced biological and biochemical analyses. Internationally established scores will be used in the three centres. Comparison of all these measurements between the two populations is performed at baseline (T0) and at 12-month follow-up (T1). Biomechanical analyses include multi-segment foot kinematics with stereophotogrammetry, plantar pressure with instrumented platforms and also 3D reconstructions of foot bones, tissues and calcifications from most modern CT scans in weight-bearing.

The primary objective is to compare the clinical and metabolic condition together with the biological and biochemical biomarkers in the two populations. Secondary objectives are (a) creation of a database for reference on metabolic conditions and structural and dynamic alterations of the diabetic foot in correlation with risk of ulceration; (b) comparison of the measurements at T0 and T1 of all the measurements; and (c) analysis of possible correlation between biomechanical parameters and the onset of ulceration in the first population. In other words, possible novel biomechanical and biochemical based biomarkers for the risks of ulceration are searched.

Ethics and dissemination The present study wants to contribute in a possible future limitation of the incidence of foot ulcerations in patients with diabetes mellitus, through careful screenings, risk stratifications and thorough monitoring. The study finally combines traditional assessments with modern multi-instrumental measuring techniques being supported by a multidisciplinary investigative approach.

The present study protocol has been recently approved by the local ethical committees ('Comitato Etico di Area Vasta Emilia Centro – AVEC', and 'Comitato Etico della

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a no-profit, multicentre, national, clinical trial for the prevention of the complications of the diabetic foot in two populations of patients with diabetes mellitus type-2.
⇒ One population has no previous history of foot ulceration, but has a moderate ulcerative risk (IWGDF grade 2); the other population has a history of foot ulcers (IWGDF grade 3) that have been healed for at least 6 months.
⇒ The three centres provide comprehensive clinical, metabolic, biomechanical, functional, biological and biochemical data to identify possible biomarkers.
⇒ These multi-instrumental multidisciplinary assessments are performed at baseline and at 12-month follow-up.
⇒ Biomechanical measurements are objective but imply inherent complexity and possible artefacts.

Romagna – CEROM'). Written informed consent will be collected for all the participants. Findings of this study will be disseminated through peer-reviewed publications and conference presentations, as always and frequently done by the present authors.

Registration details Clinical study 'Strategie multifattoriali per la prevenzione dei rischi di ulcerazione in pazienti affetti da piede diabetico', prot. DARE-DiaFoot, promoted by Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Ortopedico Rizzoli:

- CE-AVEC n° 476/2024/Sper/IOR, Principal Investigator Prof Lisa Berti, Istituto Ortopedico Rizzoli.
- CE-AVEC n° 105/Sper/2025/AOUBo, Principal Investigator Prof Uberto Pagotto, AOUBo.

Clinical study 'Strategie multifattoriali per la prevenzione dei rischi di ulcerazione in pazienti affetti da piede diabetico', prot. DARE-DiaFoot, promoted by Istituto Ortopedico Rizzoli, CET-CEROM, Reg. sperimentazioni n. 3875, Prot. 851/2025 I.5/5, local Principal Investigator Luca Dalla Paola, UO Piede Diabetico, Maria Cecilia Hosp, Cotignola (Ravenna)

Trial registration number NCT07021222.

INTRODUCTION

Diabetes mellitus (DM) constitutes a global epidemic affecting approximately 530 million people worldwide, with a forecast of up to 600 million patients by 2045; thus, it represents a heavy burden for healthcare systems. The foot and locomotion are severely affected and the costs for associated treatments are enormous. Complications such as neuropathy, vasculopathy and foot deformities increase the risk of ulceration.[1–5] In order to possibly support prevention and early and personalised treatments, specific multi-instrumental measurements (plantar pressure, kinematics, clinical data, images and radiographic scans of the foot) can now be performed in association with more traditional metabolic analyses,[6] also those conducted with modern inflammatory biomarkers.

The diabetic foot (DF) is a serious complication associated with DM type-2 and presents with deep tissue lesions mixed with neurological disorders and peripheral vascular diseases (eg, calcifications) of the lower limbs. It is estimated that approximately 15–25% of patients with DM will develop a DF ulcer during their lifetime. According to reports of the Italian National Institute of Statistics (ISTAT, 2020), DM diagnosed in Italy affects approximately 5.9% of the population, therefore approximately 3.5 million people; as the number of newly diagnosed patients with DM increases every year, the incidence of DF ulcer is also expected to increase.[7 8]

Not much is reported in the literature about the costs for DF care,[9] but recent public information reveals that the average annual direct expenditure for DF care in the USA is US$8,659 per patient. In the UK, the National Health Service spends £935 million per year, equivalent to 1% of the entire budget. In Italy, DF represents a significant component of healthcare expenditure: alone, it absorbs 12–15% of the economic resources allocated for treatments for patients with DM. 15% of these patients will suffer from an ulcer, others will undergo a leg amputation, which in 70% of cases is preceded by an ulcer. This is also associated with a high degree of morbidity and mortality within 5 years of its onset, which is 2 to 4 times higher in subjects with DM type-2 with foot complications. Unit costs for ulcer management can vary from € 4700 to over € 40 000 per year if the subject undergoes a subsequent amputation.

In this scenario,[10] it is fundamental to establish: (a) all possible risks and predisposing factors, for the patients to know these, and possibly to strengthen him/herself protective behaviours; and (b) the critical conditions of the foot and of the overall functional status as a result of the disease, for the patients to improve the self-care, in general and in particular of the foot. A long-term global scope should be limiting the incidence of foot ulcerations in patients with DM, through careful screenings, risk stratifications and thorough monitoring, finally combining also modern instruments and measuring techniques.

In order to improve prevention and to limit as much as possible critical conditions of the foot when affected by DF,[10] it seems very valuable to correlate standard clinical data and other known predisposing factors, for example with cutting-edge functional and medical imaging measures,[11] for the identification of possible new biological and biomechanical biomarkers. Some relevant preliminary studies by these authors have already shown the potentials of this synergic approach, by using instrumented gait and plantar pressure analyses, as well as CT.[12–14] These measures will allow for better tracking of the progression of the pathology and for identifying in depth and quantitatively possible signs of DF complications. Instruments and techniques for all these measures are now all available in a unique way at the premises of some of the present authors.

Whereas gait patterns[15] and plantar pressure[16] have been analysed for decades in patients with DM, the modern cone-beam computed tomography (CBCT) now does support a careful 3D reconstruction of the skeletal architecture of the foot, finally in weight-bearing.[17–21] This loading condition represents those experienced by the foot during daily living activities; using this novel technology, a number of clinical populations have been already assessed in upright posture,[22 23] which can now be used for comparisons. Starting from CBCT scans, in a few minutes 3D reconstructions of the skeletal model of the foot can be obtained, together with the exact orientation of each single bone and the location of anatomical landmarks.[19 24 25] From the latter, thickness of plantar tissues and joint relations can be worked out; even international recommendations in this respect are now proposed.[26]

A number of these clinical issues can now be addressed within a large Italian project funded by the Italian Complementary National Plan PNC-I.1 'Research initiatives for innovative technologies and pathways in the health and welfare sector' (Ministry of University and Research). The project, called 'DARE - DigitAl lifelong pRevEntion', started in December 2022 and has a programme of 4 years. The DARE project is expected to develop new products and tools, to innovate health prevention on a digital base, and also to build up a conscious, distributed, connected and multidisciplinary community able to drive

this innovation also after its completion. These goals will be achieved through basic research, and in-silico, in-vitro and in-vivo trials. A public-private partnership (with universities, national research institutions, research hospitals—ie, IRCCS, local health authorities and companies) is aimed at studying, coordinating and implementing innovative digital health solutions in Italy, addressing also relevant infrastructure, management, ethics, technical, sustainability and legal related issues (Spoke 1). A central Hub based in Bologna coordinates research activities in the area of primary (Spoke 2), and secondary and tertiary (Spoke 3) prevention, thus contributing from mitigation of health risks to disease monitoring. The present study is the task 4.2 in Spoke 3. The three institutes here involved are committed with their own staff, instruments and budget.

METHODS AND ANALYSIS

Primary and secondary objectives and trial design

This is an interventional clinical trial, without medications or medical devices.

The primary objective of this study is to compare the clinical and metabolic condition and the biological and biochemical biomarkers in two populations under observation: a Population-I of patients with DM type-2 and mild to moderate risk of ulceration at the foot; a Population-II of patients with DM type-2 with DF and a history of ulcerative lesions.

The secondary objectives are:

- Creation of a database for reference on metabolic conditions and structural and dynamic alterations of the DF in correlation with risk of ulceration.
- Comparison of all the measurements at baseline (T0) and at 12 months (T1): those clinical/metabolic (CLIN-META), biological/biochemical (BIOL-BIOC) and biomechanical/functional (BIOM-FUNC).
- Analysis of possible correlation between the latter, that is, BIOM-FUNC, measurements and the onset of ulcers in Population-I.

Study setting

The clinical trial is no-profit, multicentre, national. Promoter and coordination centre is the IRCCS Istituto Ortopedico Rizzoli (IOR). Two are the clinical populations assessed (see also the dedicated section here below): Population-I is recruited among patients with DM type-2 seen at the Centre of Diabetology in Ospedale Sant'Orsola in Bologna (Azienda Ospedaliero Universitaria (AOU), first enrolling centre); Population-II, baseline, is recruited at the Department of Diabetic Foot at Maria Cecilia Hospital (MCH) in Cotignola—RA, among those patients with DM type-2 with also DF, that is, with ulcerative lesions. The latter centre, that is, MCH, in its laboratories has the competence and the instruments to receive and to analyse also blood samples from AOU. In other words, AOU collects CLIN-META data from Population-I, MCH collects CLIN-META data from Population-II and BIOL-BIOC data from both populations. IOR collects BIOM-FUNC data in Population-I.

Patients enrolled in Population-I receive in AOU a careful CLIN-META examination in particular with established clinical and functional scoring systems. A blood sample is also taken to be sent to MCH for BIOL-BIOC analyses. These patients are then sent to IOR for BIOM-FUNC evaluations, which imply: instrumented 'Gait-analysis' of the foot and lower limbs,[27] a CBCT scan in weight-bearing,[25] and a baropodometric analysis with instrumented insoles and a platform,[12] together with a clinical evaluation of the foot including also relevant clinical scoring systems.[13] The clinical exam in AOU and the baropodometric analysis in IOR are repeated at 12-month follow-up, to check whether possible alterations associated with the risk of foot ulceration are in progress.

Also, patients in Population-II receive in MCH a careful CLIN-META examination, with the same clinical and functional scoring systems used in AOU. A blood sample is also taken to receive BIOL-BIOC analyses in MCH. These patients are not sent to IOR for BIOM-FUNC evaluations.

Patients and public involvement

Patients have not been directly involved in designing the objectives of the study. Yet, the need for this study was inspired by the interaction with patients, collecting their experiences with the disease, and trying to improve their awareness of all possible risks and predisposing factors for DF. With a better knowledge of the critical DF conditions, possibly their protective behaviours can be strengthened, and self-care can be improved.

The two patient populations

Population-I. These are patients with DM type-2 recruited in AOU, among those with no previous history of foot ulceration but with a moderate ulcerative risk: grade 2 according to the IWGDF classification.[28] Population-I receives all three groups of analyses: (a) CLIN-META in AOU, (b) BIOL-BIOC in MCH and (c) BIOM-FUNC in IOR; those not participating for any reason in any of these three analyses will be removed from the clinical trial.

Population-II. These are patients with DM type-2 recruited in MCH, typical adult subjects affected by DF, who had foot ulcers and therefore in risk grade 3 according to the IWGDF classification.[28] The ulcerative lesions must have been resolved for at least 6 months, so that there is no longer an acute inflammatory process in progress at the time of blood sampling.

In this population, the CLIN-META and BIOL-BIOC profiles are investigated, to be compared with those in Population-I, to possibly discern differences or analogies for the parameters analysed. The final scope remains the possible identification of new blood biomarkers of inflammation and/or atherothrombosis particularly those associated with the evolution of the ulcerative process.

This Population-II is assessed also at 12-month follow-up, to confirm or not the stability of the parameters shown in the blood samples collected at time 0. Also

those patients who have developed a recurrence of ulcer and not showing typical signs of the healing process will be assessed at the follow-up.

These two study populations differ in clinical characteristics, recruitment settings and locations, laboratory assessments, and thus appear to introduce confounding factors that may limit their comparability. However, the populations were intentionally defined as distinct. Population-II, recruited at MCH and classified as IWGDF risk category 3, serves as a reference group for the less severe conditions observed in Population-I, recruited at AOU. The primary objective in fact is to identify, through comparison of the two populations, those metabolic, biological or biochemical biomarkers capable of predicting worsening of the DF conditions.

Eligibility criteria

Patients will be recruited according to the following criteria, divided as for the two different populations.

Inclusion criteria

Population-I (recruitment in AOU)

- Male and female patients aged between 18 and 75 years.
- Patients with DM type-2 with mild to moderate risk of ulceration at the foot (grade 2 according to IWGDF classification).

Population-II (recruitment in MCH)

- Male and female patients aged between 18 and 75 years.
- Patients with DM type-2 and DF with a history of ulcerative lesions (grade 3 according to IWGDF classification), healed for at least 6 months.

Exclusion criteria

Population-I (AOU)

- History or evidence of ulcers at the foot.
- Infections in progress, ongoing neoplasms.
- Immunosuppressive therapies and cortisone-based therapy.
- Pregnancy and lactation.
- Inability to provide informed consent.

Population-II (MCH)

- Infections in progress, ongoing neoplasms.
- Immunosuppressive therapies and cortisone-based therapy.
- Pregnancy and lactation.
- Inability to provide informed consent.

Outcomes: CLIN-META (data collected in AOU and MCH)

Data and parameters to be measured and analysed in the present multicentre study are from three different sources and collected in combination by the three centres (see figure 1).

The CLIN-META evaluation of patients with DM will first of all include the general clinical data of the patient obtained, as far as AOU is concerned, from the METACLINIC electronic record within Diabetology. The parameters detected concern the patient's age, the disease's duration, the drug therapy in progress, any comorbidities and morphometric parameters (body mass index, height, weight, waist circumference) and brachial blood pressure. In addition, patients who refer to routine diabetes check-ups have a series of indicators useful for the visit in the 'laboratory tests' session, including:

- Glycated haemoglobin (%+mmol/L).
- estimated Glomerular Filtration Rate (mL/min).
- Blood glucose (mg/dL).
- High-Density Lipoprotein+Low-Density Lipoprotein cholesterol.
- Triglycerides.
- Creatinine (mg/dL).
- Microalbuminuria (mg/L).
- Glomerular filtrate (mL/min).
- Sodium (mmol/L).
- Potassium (mmol/L).
- Calcium (mmol/dL).
- Nitrogen (mg/dL).

During the clinical evaluation of the foot, an objective examination will be performed, which includes the detection of possible deformities, problems affecting the skin and skin appendages, the presence of functional overloads (the result of which is the formation of hyperkeratosis), the presence of sloping oedema and palpation of the peripheral pulses.

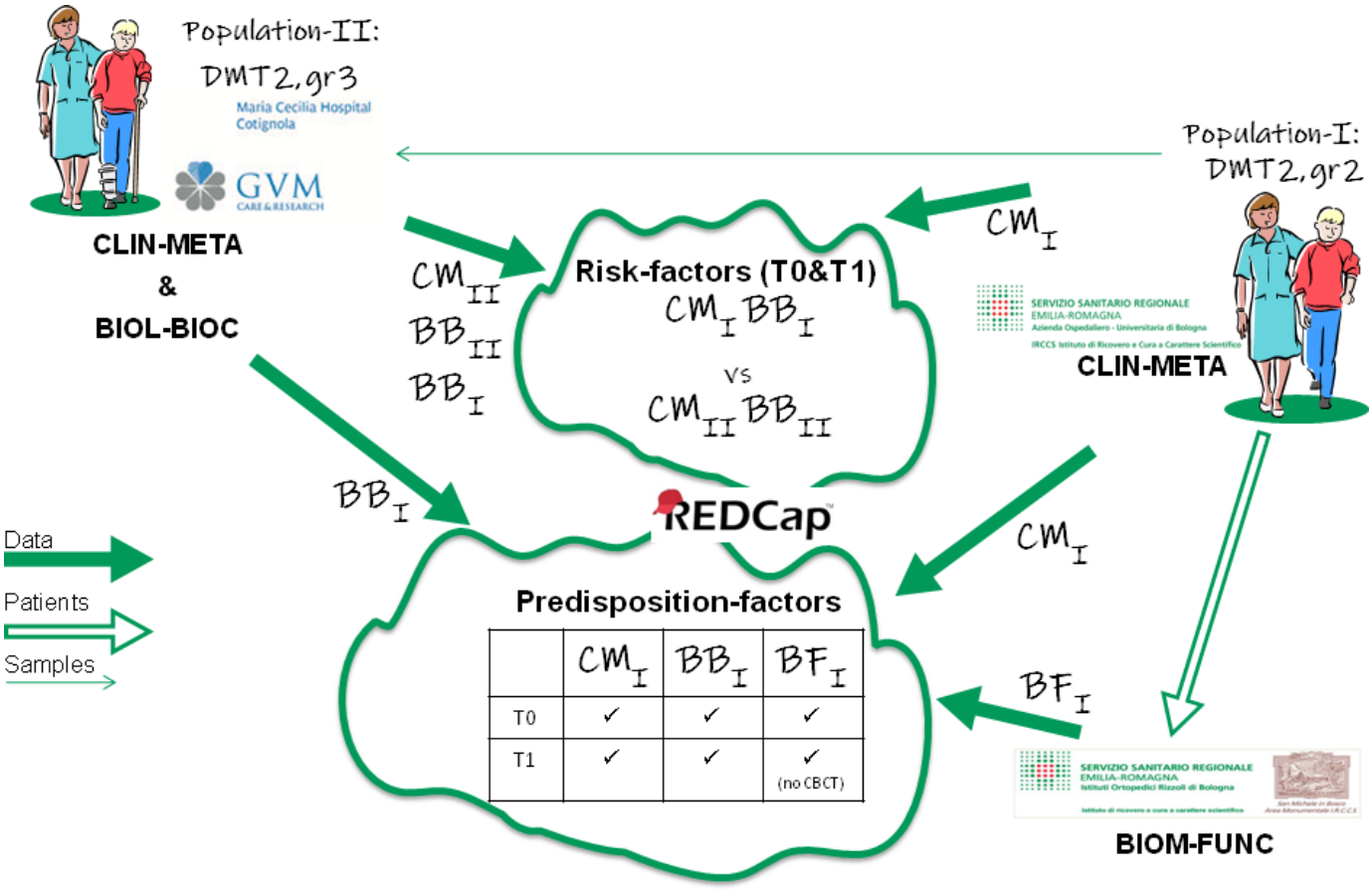
As regards the screening of the DF with relative stratification of ulcerative risk, for Population-I only, the Michigan Neuropathy Score Index questionnaire is performed with targeted questions to be submitted to the patient. Then tests are carried out to verify the presence of peripheral neuropathy (Tuning fork/Biothesiometer and 10 g Semmes-Weinstein monofilament) and evaluation for peripheral vasculopathy by ankle brachial index (ABI) and also possible presence of intermittent claudication are performed. Data related to metabolic status and neuro-ischaemic conditions will be extracted from the patient's standard medical record and its source documents.

Outcomes: BIOL-BIOC (data collected in MCH)

Parameters related to the profile of blood biomarkers for inflammation and/or atherothrombosis[29 30] will be acquired at MCH from samples collected at MCH for Population-II and from samples collected at AOU for Population-I during the CLIN-META evaluation of Population-I. In the MCH laboratories, the BIOL-BIOC evaluation of the samples will extract the following parameters, grouped as follows:

Cellular origin of microvesicles (MV)

- MV (events/microlitre).
- Anti-Cluster of Differentiation 41 (events/microlitre) (platelet-derived).
- Anti-CD41/PSel (events/microlitre) (activated platelet-derived).



	CM_I	BB_I	BF_I
T0	✓	✓	✓
T1	✓	✓	✓ (no CBCT)

Figure 1 Diagrammatic sketch of the overall workflow, with indications of the paths for patients, blood samples and data for the analyses conducted in the three centres: Maria Cecilia Hospital (MCH—top left), Ospedale Sant'Orsola (AOU—top right) and Istituto Ortopedico Rizzoli (IOR—bottom right). Each centre is responsible for either clinical/metabolic (CLIN-META, or CM), biological/biochemical (BIOL-BIOC, or BB) and biomechanical/functional (BIOM-FUNC, or BF) data; subscripts I and II are for the relevant population. Populations I and II are Diabetes mellitus Type 2 Grade 2 (DMT2, gr2) and Diabetes mellitus Type 2 Grade 3 (DMT2, gr3). Baseline (T0) and 12-month follow-up (T1) are also indicated. CBCT, cone-beam computed tomography

- PSel (events/microlitre) (activated platelet-derived).
- Anti-CD 45 (events/microlitre) (leukocyte-derived).
- Anti-CD146 (events/microlitre) (endothelial-derived).
- Anti-glycophorin A (events/microlitre) (erythrocyte-derived).
- Annexin V (AnV) (events/microlitre) (procoagulant MV).
- Anti-TF (events/microlitre).
- Anti-TFPI (events/microlitre).

Cytokines

- IL-1b (pg/mL).
- IL-6 (pg/mL).
- IL-8 (pg/mL).
- TNF-alpha (pg/mL).
- MCP-1 (pg/mL).
- MIP-1a (pg/mL).

Other biochemical parameters

- Fasting blood glucose (mg/dL).
- Glycated haemoglobin (% and mmol/L).
- Creatinine (mg/dL).
- Glomerular filtration rate (mL/min).
- Blood urea nitrogen (mg/dL).

The collection and analysis of blood biomarkers play a crucial role in understanding inflammation and atherothrombosis. The following procedures outline the methodologies employed to ensure accurate and reliable data collection.

Venous blood sampling and plasma preparation for microvesicle and cytokine analysis

Venous blood sampling is performed from the antecubital vein using a G19 needle, ensuring minimal platelet activation by removing the tourniquet after needle insertion. For the analysis of MV and cytokines, three blood collection tubes containing 0.129 M sodium citrate anticoagulant (volume 2.7 mL; BD, catalogue number: 363079) are used. To maintain the optimal blood-anticoagulant ratio, the tubes are completely filled, gently inverted three times, and processed within 30 min of collection.

All blood tubes undergo centrifugation at 2500×g for 15 min at room temperature without braking (or with minimal braking if necessary) in accordance with International Society on Thrombosis and Haemostasis

guidelines. This process separates the plasma (supernatant) from the cellular components. The plasma from the three tubes is pooled into a single tube and subjected to a second centrifugation at 2500×g for 15 min at room temperature without braking. The resulting supernatant, known as platelet-free plasma (PFP), is then divided into 300 µL aliquots and stored at −80°C for further analysis.

Microvesicle analysis

The study of circulating free MV is conducted using flow cytometry on PFP samples that have been prepared and stored at −80°C. For optimal analysis, the PFP is diluted 1:4 in a working buffer (pH 7.4), previously filtered with a 0.1 µm pore membrane. To differentiate intact MVs from cellular debris, the samples are incubated with Calcein AM (10 µM, 30 min at 37°C).

To identify the cellular origin of MVs, saturating concentrations of specific antibodies are added, including anti-CD41 (platelet-derived), anti-CD41/PSel (activated platelet-derived), anti-CD45 (leukocyte-derived), anti-CD146 (endothelial-derived) and anti-glycophorin A (erythrocyte-derived). Additional markers, such as Annexin V, anti-TF and anti-TFPI, are used to characterise procoagulant MVs. To ensure accuracy, all antibodies are centrifuged at 17 000*g* for 30 min at 4°C before use to eliminate potential aggregates.[29]

Biochemical parameters

The evaluation of inflammatory biomarkers is conducted using commercial ELISA kits. To maintain the integrity of the study, all immunoenzymatic assays are performed by laboratory staff under blinded conditions, ensuring that patient data remains confidential. The cytokines assessed include IL-1b (interleukin-1beta), IL-6, IL-8, TNF-alpha (tumour necrosis factor-alpha), MCP-1 (monocyte chemoattractant protein-1) and MIP-1a (macrophage inflammatory protein-1 alpha).

Other biochemical parameters

In addition to cytokine analysis, other biochemical parameters are assessed to determine the metabolic profile and renal function of subjects. These parameters include glucose, glycated haemoglobin, creatinine, blood urea nitrogen and glomerular filtration rate. Standard laboratory tubes and procedures are employed at the time of sample collection to ensure consistency and accuracy in the analysis of these biological markers.

Through these rigorous procedures, this part of the study aimed at providing a comprehensive understanding of inflammatory and thrombotic biomarkers, contributing to advancements in medical research and patient care. IL-6 was selected as the reference variable for sample size calculation because it represents a well-established and biologically plausible marker of systemic inflammation, particularly relevant to the pathophysiology of DM and DF complications. In addition to standard clinical examination, ABI and possible symptoms of claudication are also used but only as screening and descriptive variables. This vascular assessment was defined and agreed among the three clinical centres involved, specifically with the perspective to identify predictors of DF complications.

Outcomes: BIOM-FUNC (data collected in IOR)

BIOM-FUNC data are collected and analysed in IOR, from Population-I which is recruited in AOU and coming from three different measurement technologies. The first is thorough instrumental Gait Analysis, which incorporates the calculation of spatio-temporal parameters, multi-segment kinematics of the foot (including 3D rotations at the ankle complex, Chopart, Lisfranc and first metatarso-phalangeal joints) and the surface electromyography (ie, EMG).

The second assessment consists of CBCT scans, performed barefoot in one-leg upright posture (figure 2), thus in weight-bearing. From these scans, the following data are extracted or calculated[19 24 25]: 3D models of the foot bones; bones' inclination angles in the three anatomical planes and their relative orientation (particularly at the ankle complex and at the metatarso-phalangeal joints); and bone-to-ground distances, from which the thickness of the plantar soft tissues is estimated. Volumes of major vascular calcifications are also calculated.

A third is Baropodometry, which measures during the stance phase of gait the areas, peaks and the integral of plantar pressure, together with the so-called isometric foot strength.[31]

These three measures are taken together with traditional scoring systems for the functional assessment of the foot, and include: Visual Analogue Score (VAS), range of motion (ROM) at the ankle and first metatarso-phalangeal joints, the varus/valgus angle of the calcaneus and of the hallux, Foot Posture Index,[32] Inlow's 60-second Diabetic Foot Screen[33] and Foot Health Status Questionnaire.[31]

The measurements and parameters within BIOM-FUNC data are worked out by using the following techniques and instruments.

Gait analysis

Patients undergo instrumented non-invasive gait analysis in the IOR Movement Analysis Laboratory. Relevant instruments are a stereophotogrammetric system with nine infrared TV cameras (Vicon, Nexus motion-capture Software V.2.12.1, B10 Bonita, Oxford, UK), two force platforms (Kistler Instrument, AG Switzerland) and a 16-channel EMG system (Cometa, Milan). The kinematics analysis is performed with spherical skin markers positioned in anatomical landmarks according to the established experimental protocol 'Rizzoli-Foot-Model'[27 34 35]; with this protocol, 3D position and orientation of the shank, the rear-, mid- and fore-foot, and of the first phalanx, as well as relative orientation at the corresponding joints, are tracked. In addition to this three-planar joint motion, kinetics data are also measured, such as the three components of the ground reaction force and the plantar pressure. Using surface EMG, bipolar electrodes are positioned on the muscular belly of the main

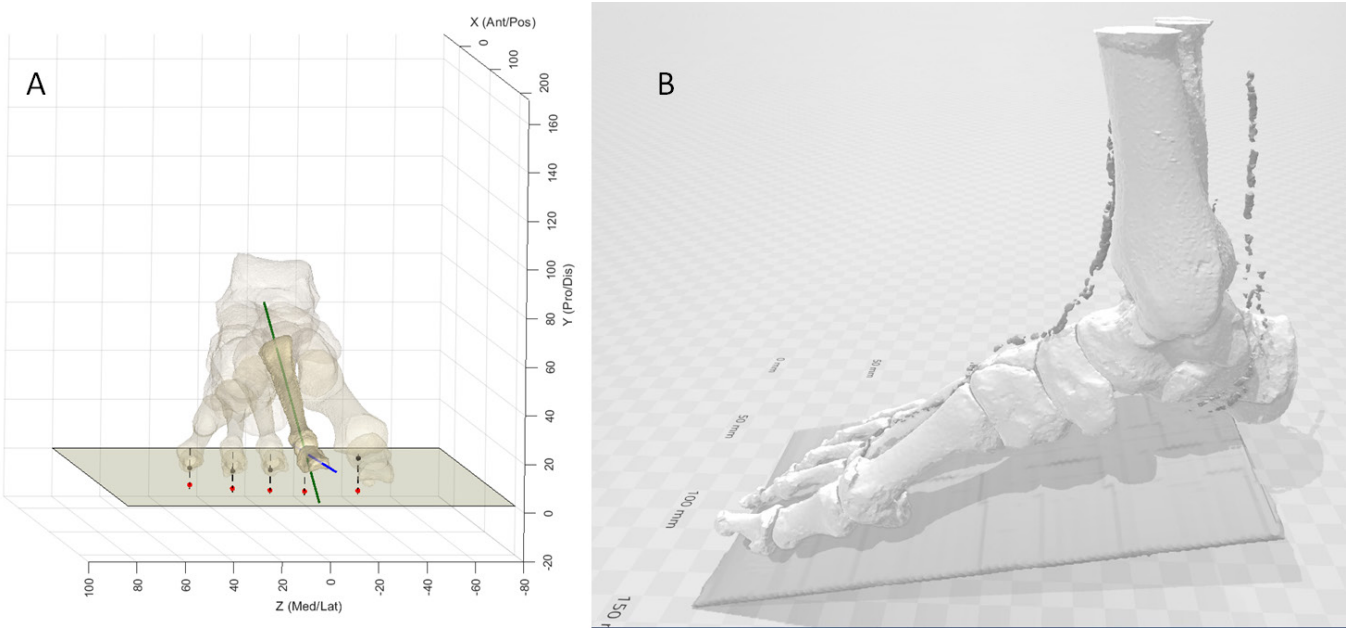


Figure 2 Representations of major measurements from 3D skeletal models of the foot, of exemplary patients with diabetic foot. (A). The longitudinal axes of the second metatarsal (green) and phalanx (blue) are depicted in these bones (all five rays are thus calculated), from which bone inclinations and joint angles can be worked out; distances from the floor are also shown, with the most plantar landmark of the distal metatarsals (black dot), and its corresponding projection on the ground plane (red dot). (B) Depiction of locations and volumes of major vascular calcifications, superimposed on the foot bones.

muscles of the distal lower limb (tibialis anterior, medial gastrocnemius, soleus and peroneus longus), according to international recommendations and guidelines (Surface Electromyography for the Non-Invasive Assessment of Muscles). Eventually, the following parameters are extracted from Gait Analysis: spatio-temporal parameters (such as progression speed, step length, percentage of stance phase), the multi-segment foot kinematics (rotations in the three anatomical planes—sagittal, coronal and transverse—of these joints: ankle, Chopart, Lisfranc and first metatarso-phalangeal), the three components of the ground reaction force (vertical, medio-lateral and antero-posterior) and also surface EMG (of the tibialis anterior, medial gastrocnemius, soleus and peroneus longus).

CBCT based analysis

By using the unique CBCT technology, scans are taken in weight-bearing from patients in Population-I using the 'OnSite 3D Extremity' (Carestream, USA). This device was designed for CT scans in upright postures of the lower and upper limbs with low doses and has been used for years in IOR for research and clinical studies.[19 20 22–25] This finally allows CT scans of segments and joints in physiological loading conditions. A few seconds after the scan a 3D rendering of the voxels is accessible; also, traditional TC images can be then navigated in high resolution along any selected anatomical axis. The emitted radiation dose is smaller than conventional CT devices (see also[17 18]). The acquisition time when the patient must be upright still is 25 s. The slice thickness of the exported file in conventional Digital Imaging and COmmunications in Medicine (DICOM) format can be adjusted a-posteriori, according to the purpose, either for diagnostic or research scopes; for the present study the 0.26 mm thickness is used. With this CBCT system, from these DICOM files, very accurate 3D bone models are reconstructed, as demonstrated by a number of recent papers from IOR researchers.[19 22–25] From these 3D bone models the following parameters are calculated, well representing the foot skeletal morphology: spatial inclination of metatarsals and phalanges, metatarso-phalangeal joint angle projections in the three anatomical planes, the bone-to-ground distance (thus the thickness of the plantar tissues). More recently an algorithm has been developed and tested to work out the aggregated volume of vascular calcifications starting from the DICOM files. This overall activity and analysis is not yet part of normal clinical practice, it is thus to be considered study-specific.

Baropodometry

Baropodometric analysis is performed by using a plantar pressure platform (P-walk, BTS, Milano) and instrumented insoles (Pedar, Novel, Germany). The contact of the plantar foot during stance is mapped with these instruments using a high spatial-resolution matrix of pressure sensors, non-invasive for the patient. The digital instantaneous footprint results in a number of pressure parameters, for the entire foot but also for eight different sub-areas; the so-called centre of pressure excursion index is also calculated. The baropodometric assessment allows to measure instantaneously the load distribution under the foot in the stance phase of gait, thus to identify possible areas of peak pressure, which are known

to be responsible for complications to the foot.[12 36 37] From these direct measurements, a number of typical baropodometric parameters are calculated and analysed: exact areas of contact, amplitude and location of peak pressure, pressure integral.

Also the following score systems are taken from the patients and analysed: VAS, ROM at the ankle and first metatarso-phalangeal joints, varus/valgus angle of the calcaneus and of the hallux, Foot Posture Index,[32] Inlow's 60-second Diabetic Foot Screen,[33] and Foot Health Status Questionnaire.[31]

These three instrumental analyses (Gait Analysis, CBCT, and Baropodometry) and relevant extractions of meaningful biomechanical parameters, together with the functional scores as here above, are all performed in IOR shortly after each CLIN-META assessment in AOU.

Follow-up (T1)

For *Population-I,* at 1 year follow-up, the CLIN-META assessment is repeated in AOU (including also the blood sampling and delivery to MCH); shortly after that, the baropodometric assessment and the score based clinical and functional assessment is performed in IOR (not the CBCT).

Population-II is also assessed (BIOL-BIOC) again at 1 year follow-up, which involves blood sampling; this to confirm whether there is stability of the results obtained at T0.

In other words, after the completion of the analyses at baseline (T0), the follow-up at 12 months is expected to reveal those patients with ulcers and/or other minor or major ailments at the foot.

Overall, the flow of patients, blood samples and data is summarised in figure 2, depicting the three centres involved and their roles and activities, together with the two populations of patients and the variables and data to be collected at T0 and T1.

Participant timeline and recruitment

All enrolled patients are recruited only after official approval of the study by relevant Ethical Committees (in our case 'CET-AVEC' for IOR and AOU, 'CET-CEROM' for MCH). The final approvals and relevant signed authorisations were received in October 2025, and the first patient was enrolled on 3 November 2025. The study is planned to be completed by May 2027.

Population-I is progressively recruited (T0) among patients with DM type-2 routinely visited at the Centre of Diabetology in Ospedale Sant'Orsola in Bologna (AOU), with ulcerative risk moderate (grade 2 according to IWGDF classification) and with no previous history of foot ulceration. After CLIN-META in AOU these are sent to IOR for BIOM-FUNC analyses, and there inserted in a digital platform (ie, SIR2020). At 12-month follow-up (T1), these subjects will be received again in AOU and IOR.

Population-II is progressively recruited (T0) among patients with DM type-2 affected by DF visited in MCH, who had foot ulcers and thus in risk grade 3 according to the IWGDF classification.[28]

Patients in both populations are routinely monitored, and any clinical outcomes such as new ulcers, infections, hospital admissions, minor and major amputations and death are recorded.

The potential candidates, both for Population-I and -II, are formally recruited for the study only after a signed informed consent as arranged by the two recruiting centres (AOU and MCH). Copy of the signed consent forms are then sent to the promoter centre (IOR). Not a single data collection and analysis is performed before the informed consent is signed.

All patients will be informed by the Principal Investigators (PI) of the aims of the study, the possible risks and benefits that can derive from the participation in the study. The PI must clearly inform patients that they are free to refuse participation in the study and that they may withdraw their consent at any time and for any reason. Patients will be also informed about the strict confidentiality of their data, but also that their medical records may be reviewed for the present trial purposes by authorised individuals other than their treating physician.

The informed consent procedure conforms to the ICH guidelines on Good Clinical Practice: this implies that 'the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative'.

The PI also signs the Informed Consent form and will keep the original at the site and a copy of the original must be handed to the patient.

The competent Ethical Committee for each Institution participating in the study has already validated local informed consent documents. It will be emphasised that participation is voluntary and that the patient is allowed to refuse further participation in the study whenever he/she wants. This will not prejudice the patient's subsequent care.

Sample size and statistical analysis

Sample size calculation was based on the biochemical 'interleukin-6 (IL-6)', a known predictive factor of inflammation, listed in the BIOL-BIOC parameters for the comparison as for the primary objective. Assuming a weighted SD of 7.9 pg/mL,[38] the study must involve 120 participants overall (with a ratio of 1:2 between the two populations, namely 40 for Population-I and 80 for Population-II) to be able to detect mean differences of IL-6 values greater than 5 pg/mL in the two populations, with a power of 90% and significance level of 5% (two-tailed test).

Population-I. Based on current volumes and considering 1 year for completion of the recruitment, this results in an estimate of 50 patients, including also a drop-out of 20%.

Population-II. Based on current volumes and considering 1 year for completion of the recruitment, this results in an estimate of at least 100 patients, including also a drop-out of 20%.

The continuous variables are analysed with mean and SD, or median and IQR according to their distribution. The categorical variables are analysed by means of frequency and percentage.

The parameters' comparison at baseline between the two populations will be based for the continuous variables on Student t-test (parametric), and on Mann-Whitney U-test (nonparametric) according to their distribution; those comparisons for the categorical variables will be based on Fisher test.

The parameters' comparison between times (T0 vs T1) will be based on a paired t-test (parametric) or paired samples Wilcoxon test (nonparametric) for those continuous variables, and on McNemar's test for the categorical variables.

Solely for Population-I, possible differences in distribution for the BIOM-FUNC parameters will be investigated, in connection with a potential worsening of the ulcerative status.

Data collection

A specific case report form (CRF) will be designed and established, to be used for data collection in each centre, both for general information, experimental data and the results of the analyses as reported above, that is, those associated with the CLIN-META, BIOL-BIOC, and BIOM-FUNC assessments. All these data will be stored in a database, in a secure server of the promoter centre IOR (ie, Fileserver 2020), and according to the digital tool REDCap. This platform is a de-facto standard for data collection in clinical trials, recommended at international levels; the study promoter IOR has a full licence and large competence. For each recruited patient a record is inserted in this database containing the data of the corresponding CRF; access to the digital database and input of CLIN-META, BIOL-BIOC and BIOM-FUNC data can be performed easily by each of the three centres even remotely. The REDCap database will be used also as an archive of all other necessary information, such as personal data, informed consent, light medical images, pictures, forms for the scores.

Once the data collection is completed as above, among the final results it is expected that the following will be available:

- Integration of all biomechanical information as derived from all those multi-instrumental 3D measures of the foot during walking, together with relevant clinical and functional scores, known to be very valuable to understand the pathophysiological condition of the DF, particularly associated with the risk of ulceration, as preliminarily addressed recently in IOR.[12–14]
- Creation of a large database of relevant values for patients affected by DF, including the metabolic status, the neuro-ischaemic conditions, blood biomarkers for inflammation, 3D skeletal reconstruction from CBCT, kinematics–kinetic–EMG analyses, plantar pressure, all related to the risk of ulceration.

ETHICS AND DISSEMINATION

The present clinical trial protocol and its documents have been approved by the competent Authorities and Ethics Committees, that is ('Comitato Etico di Area Vasta Emilia Centro – AVEC', and 'Comitato Etico della Romagna – CEROM'). The PI ensures that the study is conducted in accordance with both the most updated version of the Declaration of Helsinki and all the international and local laws which apply to clinical trials and to patient protection.

The present protocol has been written and the study will be conducted according to the principles of the ICH Harmonised Tripartite Guideline for Good Clinical Practice (ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

Privacy and confidentiality

In order to ensure confidentiality of this clinical trial, as disposed by the national and European applicable regulation, data will be accessible only to the PI and his/her designees, that is, collaborators, for the management of the study, the Ethics Committee of each Institution involved, and the Health Authority.

PI and his/her Institution will allow access to data and source documentation for monitoring, auditing, Ethics Committee revision and inspections of the Health Authority, but maintaining at all times subject personal data confidentiality as specified in the 'Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995'.

The Investigators must guarantee that patient anonymity is kept at all times and their identity must be protected from unauthorised persons and institutions. All patients included in the study will be identified with a numeric code, so that no identifiable personal data is collected (pseudo anonymisation).

The PI conserves a patients' inclusion registry where it figures the personal data of the patient: name, surname, address and corresponding identification code into the study, this register will be kept on a PI File.

Data monitoring

A central project data manager is tasked to perform data quality control on all collected data. Interim reports, every 3 months, as well as a final report, are due to the DARE Foundation, eventually to be submitted to the Italian Ministry of University and Research.

A constant monitoring of the project is performed also by the Clinical Trial Centre of the IRCCS IOR, responsible for the correct and timely progress of the Clinical Trial. A final study report is eventually due to the Ethics Committee.

Final report and publication of the results

The PI commits him/herself to produce a complete final report and to present at scientific conferences and to publish in international journals the results collected as here described in a responsible and

coherent manner. In particular, these publications will be arranged independently of the results obtained.

The dissemination of the data, via scientific talks at conferences or papers in journals, will always be supported with the results of relevant statistical elaborations, in fully anonymised formats.

Author affiliations
[1]Movement Analysis Laboratory, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[2]Prevenzione e Cura del Diabete, Azienda Ospedaliera-Universitaria Sant'Orsola (AOU) - UOC di Endocrinologia, Bologna, Italy
[3]Diabetic Foot Department, Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy
[4]Clinical Trial Centre, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[5]Medicina Fisica e Riabilitativa 1, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[6]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy
[7]Cardiovascular Department, Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy

Acknowledgements We thank Laura Maria Beatrice Belotti and Monica Cosentino for their help with the statistical planning.

Contributors AL, CB, MPL and LB designed the overall map of the study and wrote the general parts of the manuscript. AL and GS developed the figures. AL, CB, PC, GR, GS and MO contributed to the Biomechanical and Functional analyses. UP, MSG, GC and VL contributed to the Clinical and Metabolic analyses. ET, AMM, FM, ET, LF, FV and DS contributed to the Biological and Biochemical analyses. For the Italian law, the clinical and ethical guarantor is the PI of the study, LB.

Funding This research was co-funded by the Italian Complementary National Plan PNC-I.1 'Research initiatives for innovative technologies and pathways in the health and welfare sector' D.D. 931 of 06 June 2022, 'DARE - DigitAl lifelong pRevEntion' initiative, code NC0000002, CUP: B53C22006230001. SPOKE 3, WP 4, Task 4.2. Principal Investigator: Lorenzo Chiari, PhD, Department of Electrical, Electronic, and Information Engineering—Guglielmo Marconi | DEI Health Sciences and Technologies - Interdepartmental Center for Industrial Research | CIRI-SDV President | DARE Foundation - Digital Lifelong Prevention Alma Mater Studiorum - Università di Bologna, Viale Risorgimento, 2 - 40136 Bologna, Italy

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iD
Alberto Leardini https://orcid.org/0000-0003-3547-7370

REFERENCES

1 Cho NH, Shaw JE, Karuranga S, *et al*. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract* 2018;138:271–81.
2 McDermott K, Fang M, Boulton AJM, *et al*. Etiology, Epidemiology, and Disparities in the Burden of Diabetic Foot Ulcers. *Diabetes Care* 2023;46:209–21.
3 Bus SA, Sacco ICN, Monteiro-Soares M, *et al*. Guidelines on the prevention of foot ulcers in persons with diabetes (IWGDF 2023 update). *Diabetes Metabolism Res* 2024;40:e3651.
4 Schaper NC, van Netten JJ, Apelqvist J, *et al*. Practical guidelines on the prevention and management of diabetes-related foot disease (IWGDF 2023 update). *Diabetes Metab Res Rev* 2024;40:e3657.
5 van Netten JJ, Raspovic A, Lavery LA, *et al*. Prevention of foot ulcers in persons with diabetes at risk of ulceration: A systematic review and meta-analysis. *Diabetes Metab Res Rev* 2024;40:e3652.
6 Wang Y, Shao T, Wang J, *et al*. An update on potential biomarkers for diagnosing diabetic foot ulcer at early stage. *Biomedicine & Pharmacotherapy* 2021;133:110991.
7 Lo ZJ, Surendra NK, Saxena A, *et al*. Clinical and economic burden of diabetic foot ulcers: A 5-year longitudinal multi-ethnic cohort study from the tropics. *Int Wound J* 2021;18:375–86.
8 Monami M, Scatena A, Miranda C, *et al*. Development of the Italian clinical practice guidelines for the treatment of diabetic foot syndrome: design and methodological aspects. *Acta Diabetol* 2023;60:1449–69.
9 Savelieff MG, Elafros MA, Viswanathan V, *et al*. The global and regional burden of diabetic peripheral neuropathy. *Nat Rev Neurol* 2025;21:17–31.
10 Da Ros R, Volpe A, Bordieri C, *et al*. Prevention of foot ulcers recurrence in patients with diabetes: a systematic review and meta-analysis of randomized controlled trials for the development of the italian guidelines for the treatment of diabetic foot syndrome. *Acta Diabetol* 2024;61:1363–73.
11 Godavarty A, Leiva K, Amadi N, *et al*. Diabetic Foot Ulcer Imaging: An Overview and Future Directions. *J Diabetes Sci Technol* 2023;17:1662–75.
12 Caravaggi P, Leardini A, Giacomozzi C. Multiple linear regression approach for the analysis of the relationships between joints mobility and regional pressure-based parameters in the normal-arched foot. *J Biomech* 2016;49:3485–91.
13 Belvedere C, Giacomozzi C, Carrara C, *et al*. Correlations between weight-bearing 3D bone architecture and dynamic plantar pressure measurements in the diabetic foot. *J Foot Ankle Res* 2020;13:64.
14 Giacomozzi C, Lullini G, Leardini A, *et al*. Analysis of Clinical Profiles, Deformities, and Plantar Pressure Patterns in Diabetic Foot Syndrome. *Appl Sci (Basel)* 2021;11:11464.
15 Hazari A, Maiya AG, Shivashankara KN, *et al*. Kinetics and kinematics of diabetic foot in type 2 diabetes mellitus with and without peripheral neuropathy: a systematic review and meta-analysis. *Springerplus* 2016;5:1819.
16 Lockhart M, Dinneen SF, O'Keeffe DT. Plantar pressure measurement in diabetic foot disease: A scoping review. *J Diabetes Investig* 2024;15:990–9.
17 Ludlow JB, Ivanovic M. Weightbearing CBCT, MDCT, and 2D imaging dosimetry of the foot and ankle. *IJDI* 2014;1:1.
18 Ludlow JB. Hand-wrist, knee, and foot-ankle dosimetry and image quality measurements of a novel extremity imaging unit providing CBCT and 2D imaging options. *Med Phys* 2018;45:4955–63.
19 Leardini A, Durante S, Belvedere C, *et al*. Weight-bearing CT Technology in Musculoskeletal Pathologies of the Lower Limbs: Techniques, Initial Applications, and Preliminary Combinations with Gait-Analysis Measurements at the Istituto Ortopedico Rizzoli. *Semin Musculoskelet Radiol* 2019;23:643–56.
20 Lintz F, de Cesar Netto C, Belvedere C, *et al*. Recent Innovations Brought about by Weight-Bearing CT Imaging in the Foot and Ankle: A Systematic Review of the Literature. *Appl Sci (Basel)* 2024;14:5562.
21 Carrara C, Caravaggi P, Belvedere C, *et al*. Radiographic angular measurements of the foot and ankle in weight-bearing: A literature review. *Foot Ankle Surg* 2020;26:509–17.
22 Pavani C, Belvedere C, Ortolani M, *et al*. 3D measurement techniques for the hindfoot alignment angle from weight-bearing CT in a clinical population. *Sci Rep* 2022;12:16900.
23 Sacchetti G, Belvedere C, Ortolani M, *et al*. Three-Dimensional Bone Alignment from Cone-Beam Computed-Tomography Scans in Weight-Bearing and Clinical Outcomes Following the Modified Grice–Green Surgical Procedure for Adult Acquired Flatfoot. *Appl Sci (Basel)* 2024;14:8521.
24 Carrara C, Belvedere C, Caravaggi P, *et al*. Techniques for 3D foot bone orientation angles in weight-bearing from cone-beam computed tomography. *Foot Ankle Surg* 2021;27:168–74.
25 Ortolani M, Leardini A, Pavani C, *et al*. Angular and linear measurements of adult flexible flatfoot via weight-bearing CT scans and 3D bone reconstruction tools. *Sci Rep* 2021;11:16139.
26 Kruger KM, Lenz AL, Dibbern KN, *et al*. Standardizing 3 Dimensional Measurements in Foot and Ankle Imaging. *Foot Ankle Clin* 2025;30:221–37.
27 Leardini A, Benedetti MG, Berti L, *et al*. Rear-foot, mid-foot and fore-foot motion during the stance phase of gait. *Gait Posture* 2007;25:453–62.
28 Senneville É, Albalawi Z, van Asten SA, *et al*. IWGDF/IDSA Guidelines on the Diagnosis and Treatment of Diabetes-related Foot Infections (IWGDF/IDSA 2023). *Clin Infect Dis* 2023.:ciad527.

29 Camera M, Brambilla M, Canzano P, *et al*. Association of Microvesicles With Graft Patency in Patients Undergoing CABG Surgery. *J Am Coll Cardiol* 2020;75:2819–32.
30 Zhang H, Dhalla NS. The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease. *IJMS* 2024;25:1082.
31 Ferreira JSSP, Panighel JP, Silva ÉQ, *et al*. Foot function and strength of patients with diabetes grouped by ulcer risk classification (IWGDF). *Diabetol Metab Syndr* 2019;11:89.
32 Redmond AC, Crosbie J, Ouvrier RA. Development and validation of a novel rating system for scoring standing foot posture: the Foot Posture Index. *Clin Biomech (Bristol)* 2006;21:89–98.
33 Inlow S. The 60-second foot exam for people with diabetes. *Wound Care Canada* 2004;2:10–1.
34 Portinaro N, Leardini A, Panou A, *et al*. Modifying the Rizzoli foot model to improve the diagnosis of pes-planus: application to kinematics of feet in teenagers. *J Foot Ankle Res* 2014;7:754.
35 Caravaggi P, Giacomozzi C, Lullini G, *et al*. The Effect of Neuropathy and Diabetes Type on Multisegment Foot Kinematics: A Cohort Study on 70 Participants with Diabetes. *Appl Sci (Basel)* 2021;11:8848.
36 Caravaggi P, Giangrande A, Lullini G, *et al*. In shoe pressure measurements during different motor tasks while wearing safety shoes: The effect of custom made insoles vs. prefabricated and off-the-shelf. *Gait Posture* 2016;50:232–8.
37 De Blasiis P, Caravaggi P, Fullin A, *et al*. Postural stability and plantar pressure parameters in healthy subjects: variability, correlation analysis and differences under open and closed eye conditions. *Front Bioeng Biotechnol* 2023;11:1198120.
38 Mohamed AA, Elmotaleb Hussein MA, Nabil Hanna I, *et al*. The potential impact and diagnostic value of inflammatory markers on diabetic foot progression in type II diabetes mellitus: A case–control study. *Medicina Clínica* 2024;162:e33–9.